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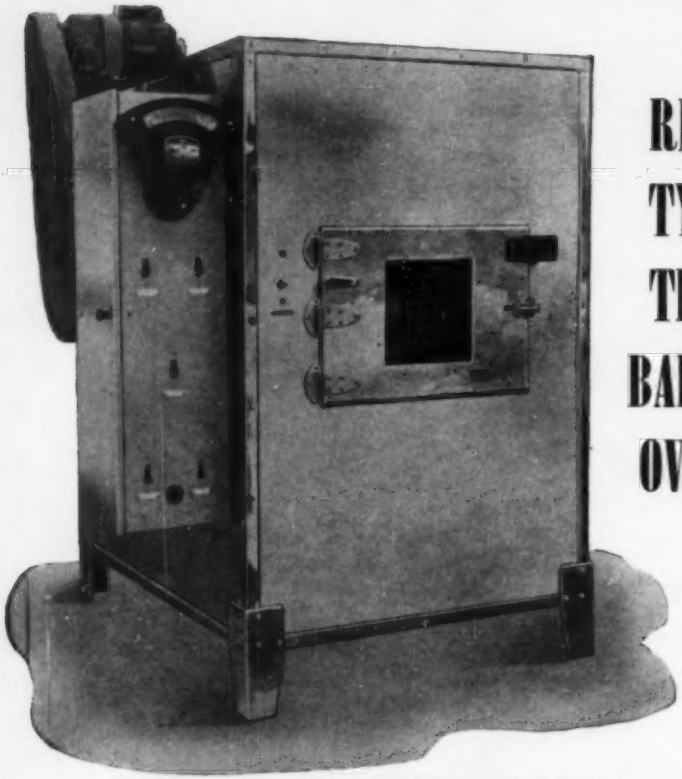

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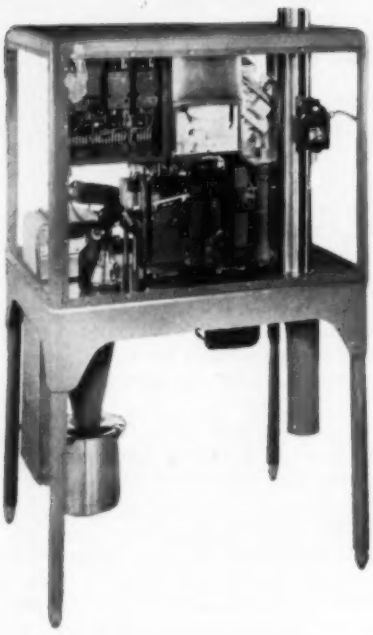

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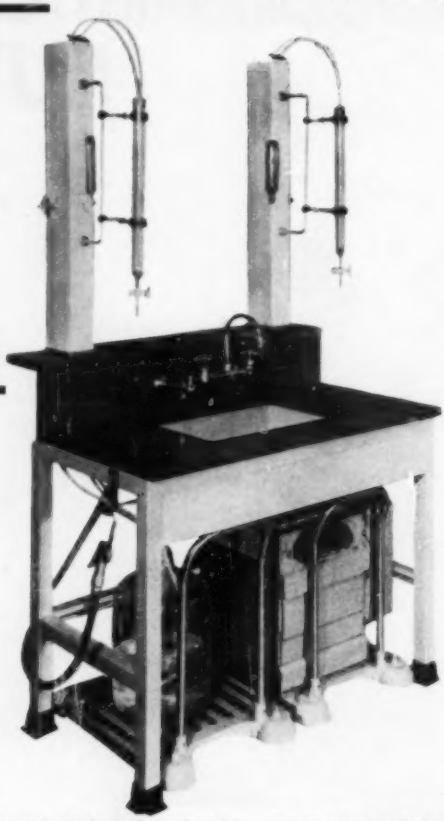

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CEREAL CHEMISTRY

VOL. 36 NOVEMBER, 1959 NO. 6

THE SUBSIEVE-SIZE FRACTIONS OF A SOFT WHEAT FLOUR PRODUCED BY AIR CLASSIFICATION[1]

REZSOE GRACZA[2]

ABSTRACT

Repeated air classification of a conventionally milled soft red winter wheat flour (56.2% extraction, flour basis) containing 7.8% protein, starting with separation at low critical particle size, yielded seven fractions containing from 22.2% protein for the fraction with the smallest particle size to 2.5% protein. Other indices such as ash, diastatic activity, alkaline water retention capacity, thiamine, fat, and MacMichael viscosity generally increased with increasing protein content. Specific gravity, bulk density, and pH increased with decreasing protein content. Farinograph and extensigraph tests showed an increase of absorption and strength properties with increasing protein content.

The relationship of protein content versus flowdynamically determined particle size of a soft wheat flour was established. Another relationship was established – namely, the protein content of coarse and fine fractions as a function of critical particle size at which the parent flour was separated in individual process steps. The intercept of this curve pair determines a particle size called the "neutral critical cut" under which individual flowdynamic separations produced fines with higher protein content than the coarse fractions. Above this "neutral critical cut" a small but reversed protein shifting was evident. For the measurement of protein shifting within the fractions of a flour, an index is proposed which designates in percentage the amount of protein in the parent flour which was shifted out or into the fractions; this index in the present study was found to be $\delta = 27.7\%$. Factors of flowdynamic size such as geometric measurement, density, and shape are analyzed.

At the turn of the last century, a milling system was adopted to produce wheat flours which made general use of roller mills and a large variety of sieves. With the passing of time, new requirements set by consumers stimulated exploration and development of new techniques and new machinery which has led to better understanding of the properties of the raw material and the flour.

The subject of flour particle size and particle size distribution of

[1] Manuscript received May 4, 1959.
[2] Address: The Pillsbury Company, Minneapolis, Minn.

flours intrigued many investigators in cereal science. One school of investigators attacked the problem by reducing the particle size of flour to progressively finer granulation. Their samples had progressively larger specific surface and progressively slightly narrower, but still not considerably different, size distribution character than their parent stocks (21). These samples were subjected to characterization by chemical, physical, and baking indices. These studies may be aptly called *size reduction studies.*

The other leading approach to the particle size problem was that the flour could be divided into groups according to particle size ranges. This approach produced flour fractions which had specific surfaces larger and smaller than their parent flour, and had narrow size ranges with considerably different size distribution characteristics than their parent stock. These studies are called *size classification studies.* This second approach was followed by LeClerc *et al.* (16) and later by Kress (15). Shollenberger and Coleman (21) followed the routes of both size reduction and size classification, and they compiled extensive chemical, physical, and baking data. Shellenberger *et al.* (20) fractionated flours with standard Tyler sieves down to 400-mesh, i.e. 38-micron opening. They used air elutriation "to remove bran chips and all other contaminating material" from the sieve fractions, and they did so with the additional purpose of "breaking of the flour agglomerates by air elutriation," thus obtaining better accuracy of particle size separation than by sieving alone.

In recent years, a large number of publications show the general interest in size grading procedures which classify flour particles only by their flowdynamic characteristics. Hanssen and Niemann (11) reported slight protein displacement due to air classification in reground and not reground German soft wheat flour producing two flour fractions. By improving equipment and procedures Hanssen and Florian (10) obtained a fine fraction with 22.3% protein content and a coarse fraction with 7.4% protein content from a German soft wheat parent flour of 9.2% protein content. Elias and Scott (7) used grinding and classification, producing three fractions out of English soft wheat and English hard wheat, where the fine fraction was high in protein content, and the medium-size fraction was low in protein content. Wichser (23) discussed some practical aspects, especially the baking properties of four commercially air-classified flour fractions. He demonstrated that fractions concentrating protein improved bread-baking capacity over parent stock, and fractions concentrating starch granules were specifically useful for pastries. Jones *et al.* (13) produced ten flour fractions in a repeated air classification of English soft wheat flour using

laboratory equipment. Their experiments include also the effect of grinding by specific equipment previous to air classification to yield a flour fraction with 2.6% protein content. These authors found yield and protein differences among the fine, intermediate, and coarse fractions of soft and hard English wheat flours. They report cut setting of machine, yield, protein, maltose, color data, and some baking results of the fractions.

The material reported here is essentially a partial repetition and a more detailed account of the different processing schemes disclosed in the specifications of a patent application (19). It is concerned with the changes in some physical and chemical properties which occur in the flour fractions if fractionated by an air-classification technique using flowdynamic principles in what is known as the subsieve size range. Properties of seven air-classified fractions are presented which were produced from American soft wheat flour in a production-size air classifier.

Materials and Methods

The parent flour used in the test series was an unbleached, untreated, 56.2% extraction (flour basis), short patent soft wheat flour milled commercially from Ohio soft red winter wheat, having 10.2% protein content and test weight of 58.0 lb. per bu. The parent flour was fractionated 5 months later. The flour received no additional grinding after the conventional soft wheat mill flow. Thus, the separations can be considered typical of what can be obtained for such soft wheat flours with the use of an air-classification technique.

The parent flour was fractionated in a commercial-size air classifier (18) which was provided with a special forced-vortex-type rotor in the classification zone similar to the type disclosed by Lykken (17). This fractionation produced seven fractions of subsieve size in six process steps out of the parent flour, analogous to a sieve fractionation where sieves are stacked upon each other. Two main differences exist in the analogy:

1. In stacked sieve procedures, the parent flour is generally sifted starting with the largest sieve opening, the finest fraction passing through sieve openings of decreasing size. In the subsieve-size fractionation, the finest particles were removed first from the parent stock and the coarsest last.

2. Whereas the fractionation by sieves generally makes use only of the size of the particles, fractionation by air classification makes simultaneous use of size, shape, and density of the particles.

The flowsheet in Fig. 1 identifies the samples and indicates the

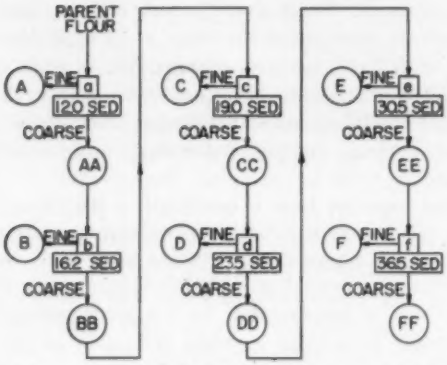


Fig. 1. Flow diagram of a repeated air-classification procedure at increasing critical particle sizes. The small letter indicates the process step; the single and double capital letters denote the fine and coarse materials, respectively.

critical cut setting of the classifier, which in crude analogy would be the equivalent of sieve openings. The parent stock was subjected to a first-stage air separation step (a), at 12 SED micron[3] critical cut, resulting in a fine fraction A and a coarse fraction AA. The first-stage fraction AA was subsequently subjected to a second-stage air-separation step (b), at a larger, namely, 16.2 SED micron critical cut, resulting in a fine fraction B and a coarse fraction BB. The repeated classification of the coarse fractions was done five times; thus the procedure resulted in seven fractions, increasing in size, namely A, B, C, D, E, F, and FF.

The detailed derivation of the critical particle size at which size graders operate and the efficiency of the size grading operation is the subject of a separate study. Since there is no precedence in flour technology for adoption of a method for determining the critical particle size and the efficiency of classification procedures, in the present work the critical cut is defined as that size at which sharpness of a classification is at maximum. Sharpness is defined as an empirical efficiency measure comparing particle size distribution of a coarse and fine fraction from an air-classification procedure. It can be obtained at an arbitrary particle size by deducting from 100% the sum of the oversize particles and undersize particles expressed as percentages of the respective fractions. Figure 2 illustrates the graphical determination of sharpness and critical cut of an air-classification procedure.

Particle size measurements were made by a slight modification of

[3] The size range of the fractions is defined by the size unit of SED micron, where the SED letters stand for Stokes Effective Diameter. Those size ranges can be expressed by other than SED micron units, such as, FD (flowdynamic) units, which include, in part, the Andreason shape factor (1.612). The following regression equation expresses the relation between the two units: $Y = 3.2463 + 0.6430\,x - 0.0013\,x^2$, where Y is SED micron and x is FD micron.

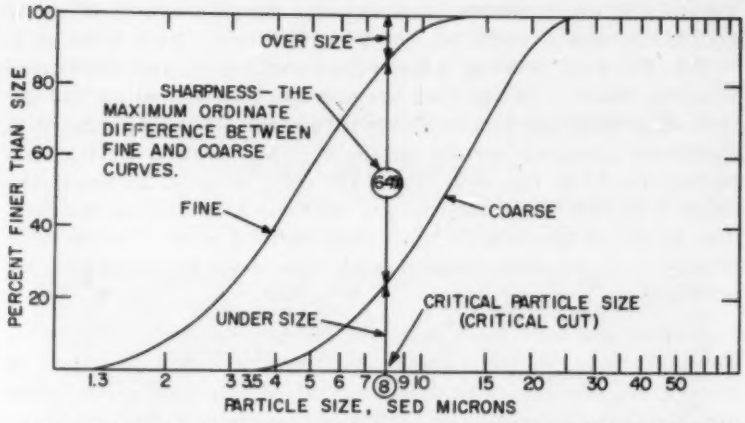


Fig. 2. An illustration of the graphical method of determining the sharpness and critical cut size from the fine and coarse material particle size distribution curves.

the method of Whitby (22), whose rapid method is based on the settling velocity of particles in the gravitational field and in the potential field of central motion. The method uses a mixture of 25% naphtha and 75% benzene (C_6H_6) by volume for dispersion and benzene for sedimentation.

The average particle diameter figures were obtained by the Fisher subsieve-sizer apparatus (8) which employs the principle of permeability of porous beds first used by Carman in liquids (5) and later by Gooden and Smith (9) in different fluids.

The following analyses were made with slight modification of the procedures identified by *Cereal Laboratory Methods* (1): protein 67.1, moisture 48.3, ash 9.1, diastatic activity 24.1, pH 60.2. Thiamine and MacMichael viscosity tests were made according to methods 98.8c and 97.1, respectively. Alkaline water retention capacity tests were run according to the method of Yamazaki (24). Fat content values were obtained with a modification of the AOAC Soxhlet extraction (4) using petroleum ether as the solvent. Specific gravity values were determined by ASTM Method C-188 (3). Color reflectance values were obtained from a kerosene-flour slurry measured on glass with a Gardner Differential Tristimulus Colorimeter. Free fatty acid content was determined by AOCS Method CA5a-40 (2).

Bulk density was determined as follows: The flour sample was sifted into a 500-ml. cylindrical steel cup (diameter 3 in.) which has a detachable collar extending its volume to about 750 ml. The cup is

then placed into a shaker consisting of a Bakelite base holding the sample cup, frame, and a Syntron Electric Vibrator (type V15B, style 1427-L, 110 volts, 60 cycle, 2.5 amps) mounted on sponge rubber and weighing about 14.35 kg. After the unit has been shaken for 120 seconds in a direction perpendicular to the axis of the cylinder with maximum amplitude setting on the controls (Syntron electric controller, type VCR 155, style 4253A, 110 volts, 60 cycle, 2.5 amps), the collar is removed from the cup and the flour is leveled and weighed. The weight of the flour in kg. multiplied by 2 is equal to the bulk density in kg. per liter. Experimental error of the test is ±0.0065 kg. per liter.

Farinograms were made using the 300-g. mixer and Method 26.4, Constant Flour Method. The dough for extensigrams consisted of flour, water, salt (2%). The dough was mixed 1 minute, rested 5 minutes, remixed 4 minutes. The absorption was varied so that after mixing the consistency was 500 B.u. ± 10 B.u. The parent flour, fractions D, E, F, and FF, because of low protein content, were remixed only 1 minute. The dough handling schedule was a stretch at 1 hour, round, mold, and rest for 1¼ hours, at which time another round-and-mold was made. After 45 minutes of rest, the second stretch was made; total elapsed time, 3 hours. The parent flour, fractions D, E, F, and FF were stretched at 1 hour only.

Amylograph tests were made with 65 g. flour (14% moisture basis) and 460 ml. of boiled and cooled distilled water.

To prepare material for microscopic measurements and the subsequent calculations of shape factor characteristics of particles of protein matter and starch granules, the following procedure was used:

An air-classified fraction rich in particles of protein matter was selected, since it had a narrow particle size range of the smallest particles and also because it contained the maximum amount of elementary flour particles, namely, particles of protein matter and starch granules. The flour was analyzed for particle size distribution by the Whitby method (22). Benzene was evaporated from the column of the sedimentation tube at room temperature. At any chosen height of the sedimentation column in the tube, particles of the same flowdynamic size, SED microns, were collected. The total column height in this case was 11 mm. At 1, 3, 6, and 11 mm., individual samples were isolated by partitioning the marked glass tube and sedimentation column at the desired height. Slides of each sample were prepared for microscopic observation using oil of 1.600 refractive

index. The largest starch granules and particles of protein matter were selected on each slide and the maximum dimensions and areas recorded. Calculations were then made to express the degree of irregularity of the protein matter and the shape relations between particles of protein matter and starch granules.

Results and Discussion

Microscopic Studies. Figures 3 and 4 are photomicrographs of the soft wheat parent flour and its seven subsieve-size fractions.

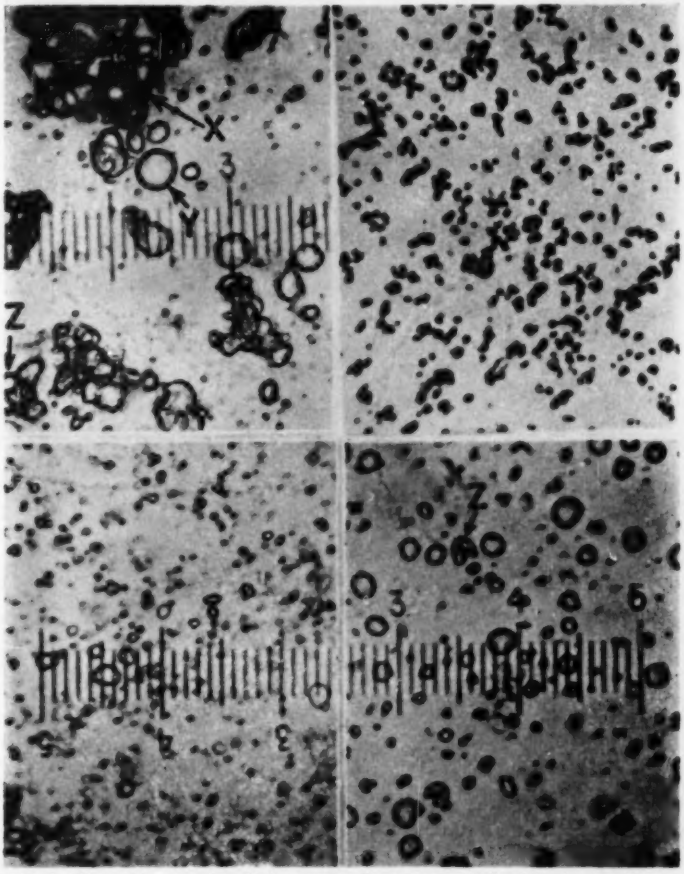


Fig. 3. Photomicrographs of the parent flour (upper left, 180×), the A fraction (upper right, 180×), the B fraction (lower left, 180×), and the C fraction (lower right, 180×). Slide prepared in an oil of 1.400 refractive index.

The photomicrographs of the parent soft wheat flour show endosperm clusters or chunks, X, up to 100 microns consisting of various sizes of starch granules cemented together by the protein matrix. The same field also contains larger starch granules, Y, some of which still carry portions of protein matter on their surface from the protein matrix of the original endosperm structure. The smallest starch granules or starch granule groups, Z, are in most cases embedded in frag-

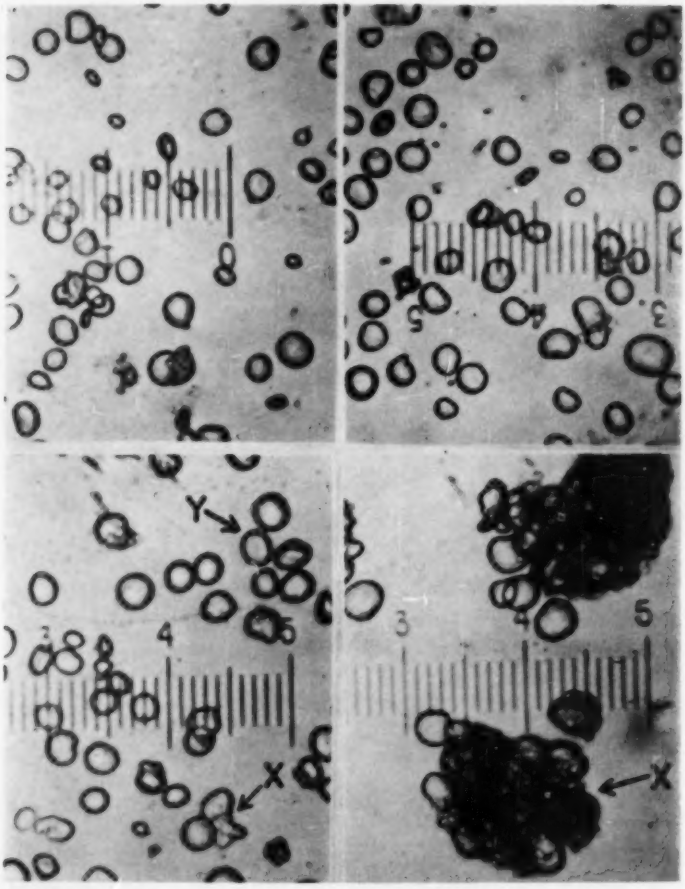


Fig. 4. Photomicrographs of fractions D (upper left, 180×), fraction E (upper right, 180×), fraction F (lower left, 180×), and the residual fraction FF (lower right, 180×). Slide prepared in an oil of 1.400 refractive index.

ments of protein matter. There are some minute fragments of the former endosperm matrix which we may identify as protein matter particles. Figure 5 is an electron microscope photograph of a particle typifying the protein material. These particles have a particular "elk-horn" shape. Their edges are indicative of the indentation or absence of starch granules which occupied the indentation voids in the protein matrix before the particles were reduced in size.

Fig. 5. Electron photomicrograph of a protein particle, magnification about 7500×.

Classification at critical cuts of smaller than 16.2 SED micron, as in process steps (a) and (b), separated out of the mixed particles of the parent flour, particles such as those shown on the photomicrographs, Fig. 3, A and B fractions. They are the smallest particles sized flow-dynamically which belong in the categories of protein matter, and the smallest starch granules or starch granule groups with a few medium-sized starch granules up to 15- to 18-micron major diameter size. The total protein matter and total starch granule content in this fraction is proportioned in such a manner that the measured protein content, N × 5.7 (14), is almost three times greater than the protein content of the parent stock. Assuming that the nitrogen content is directly proportional to the volume or weight of the protein matrix, it may be said that the protein matter "shifted" into specific fractions.

Classification step (c) concentrated particles out of the BB fraction in the +16.2 − 19.0 SED micron flowdynamic size range. As shown in the photomicrograph, Fig. 3, C fraction, this fraction included larger groups of the smallest starch granules cemented together by protein matrix (Z) and medium-sized starch granules up to 22- to 25-micron major diameter size. Some of the latter were more or less covered with

protein matter. The proportion of these particles is such that its protein content was about the same as that of the parent flour.

Classification steps (d), (e), and (f) concentrated particles out of CC fraction in the +19.0 – 36.5 SED micron flowdynamic size range. According to photomicrographs, Fig. 4, D, E, and F fractions, these particles were substantially starch granules of various sizes up to 45 microns (major diameter). In F fraction, the large starch granules (Y) and the few endosperm clusters (X) have similar sizes. These exist in such a proportion that the protein content of these fractions was about 2.5 times less than that of their parent flour. In this case it may be said that protein matter was withdrawn or "shifted" out of specific fractions.

The classification in process step (f) concentrated particles out of EE fraction which were larger than 36.5 SED micron size. The microphotograph, Fig. 4, fraction FF, lower right, reveals mainly endosperm clusters or chunks (X) larger than approximately 35–40 microns (major diameter), along with a few of the largest starch granules. The proportion of the starch granules and protein matrix material in this chunk fraction was about the same as that of the parent flour, so the protein content was also about the same.

Protein Content of the Fractions. The customary factor of 5.7 for converting Kjeldahl nitrogen values on wheat and wheat products (14) has been employed in computing the protein content of the various fractions. This is based on the arbitrary assumption that the nitrogen content of the protein in all fractions is 17.54%. The extension of this practice to isolated flour protein matter may be an arbitrary assumption. In the absence of sufficient evidence to the contrary, this assumption is used.

The histogram (Fig. 6) illustrates that protein materials are concentrated in some fractions, namely, A, B, and C, in the lower SED micron size range below about 20 SED micron size. This makes the protein content of these fractions higher than that of the parent flour. Starch granules are concentrated in other fractions, namely D, E, and F, in about the 20–37 SED micron size range. This makes the protein content of these fractions lower than that of the parent flour. The coarsest fraction, in this specific case larger than 36.5 SED micron size, has again slightly higher protein content than the parent stock. This may be attributed to concentrating the larger and probably harder endosperm fragments originating from a particular part of the wheat kernel. The figure illustrates a quantitative interpretation which could be anticipated from the microscopic evaluation.

Since the area below the base line of 7.8% protein content on Fig.

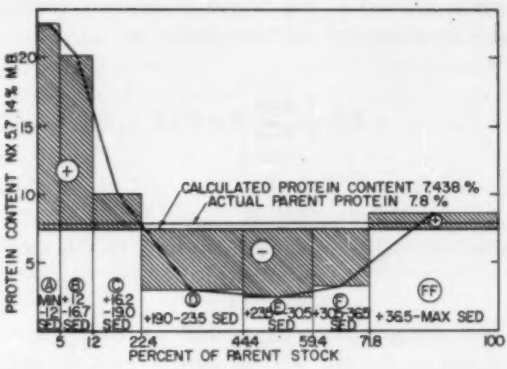


Fig. 6. Histogram of protein content vs. percent of parent stock, from the seven subsieve-size fractions of a soft wheat flour, illustrating the positive and negative protein shifting in the respective fractions.

6 is proportional to the protein content of the parent flour, the area above the parent's protein level (marked with +) will be proportional to the amount of protein increase in the fractions as related to the parent stock. If this area is expressed as the percentage of the parent's protein content, it provides a figure expressing the percentage of the parent's protein content which was shifted into the specific fractions during the classification procedure. This figure is defined as the degree of positive protein shifting, $+\delta$, which may be expressed mathematically as follows:

$$+\delta = \frac{1}{P} \sum_{x=1}^{x=n} (P_x - P)\,Y$$

where δ = degree of protein shifting (percent);
P = protein content of parent flour (percent);
P_x = protein content of individual fractions having larger protein level than the parent (percent);
P_x = protein content of individual fraction having lower protein level than the parent (percent);
Y = yield of the individual fraction (percent); and
n = number of fractions produced out of the parent stock.

A positive shift in protein of the parent stock into certain fractions can be done only at the expense of a depletion of the same amount of protein from other fractions. This area is designated by the minus sign.

This depletion is defined as the degree of negative protein shifting, $-\delta$, which may be expressed mathematically as:

$$-\delta = \frac{1}{P} \sum_{z=1}^{z=n} (P - P_z) Y$$

Therefore:

$$+\delta = -\delta = \delta$$

where δ is called the *degree of protein shifting*. In this specific case:

$$+\delta = 25\%$$
$$-\delta = 30.4\%$$

If δ is computed as the average of $+\delta$ and $-\delta$, $\delta = 27.7\%$.

The discrepancies in the $+\delta$, $-\delta$ figures may be due to errors such as:

1) Sampling error;
2) Ordinary weighing error and the absence of correction for the moisture loss;
3) Error of the analytical method for protein;
4) Absence of a correction for the slight loss in protein matter through the secondary air filter system; and
5) Limitation in the number of fractions (n).

The useful protein shifting in terms of concentration and depletion may be expressed at 2 δ. This total spread is the sum of the absolute values of $+\delta$ and $-\delta$. The *total protein spread* figure in this case would be 55.4%. In some cases it is more desirable to express the total protein spread as 2 $(-\delta)$ since this expression includes the correction for protein loss in the separation procedure.

TABLE I

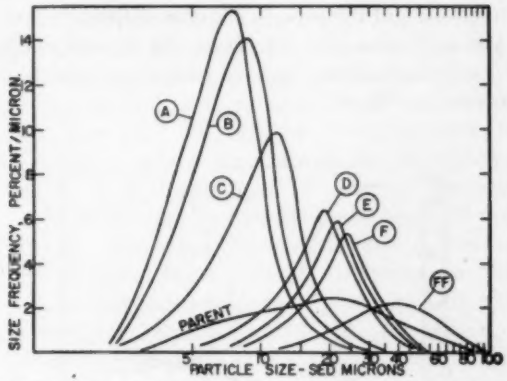
CUMULATIVE PARTICLE SIZE DISTRIBUTION DATA OF A SOFT WHEAT FLOUR AND ITS SEVEN SUBSIEVE-SIZE FRACTIONS

SIZE SED MICRONS	PERCENT FINER THAN SIZE							
	Parent	A[a]	B	C	D2	E	F	FF
100	100.0							100.0
80	98.5							94.1
60	88.8			100.0	100.0	100.0	100.0	80.6
40	69.0		100.0	99.4	97.5	98.2	91.4	38.4
30	57.4	100.0	97.6	97.2	91.2	87.6	64.8	17.3
20	33.8	98.2	93.8	86.1	58.1	11.3	6.8	3.8
10	6.3	78.2	62.5	30.5	2.9	0.0	0.0	0.0
5	3.4	49.4	37.5	12.2	0.4	0.0	0.0	0.0
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Fraction A is the first fine fraction; B through F are the fine fractions from repeated classification steps at increasingly higher critical particle size, FF being the coarse fraction of the last step.

For the ideal case where the sizes of the individual particles within an arbitrary fraction are limited by the critical cut values shown on the protein histogram, Fig. 6, the protein content may be plotted against the midpoint of critical cut limits, resulting in the *relationship between protein content and particle size* SED micron of the processed parent flour.

Table I illustrates the cumulative particle size distribution data obtained by the modified Whitby method. From these, the bell-shaped distribution curves of Fig. 7 were prepared. Presentation of the size distribution data in this fashion clarifies the division, and the overlap is easily discernible.



7. Size disribution curves of the parent flour and its seven subsieve-size fractions plotted as percent per micron vs. logarithm of size.

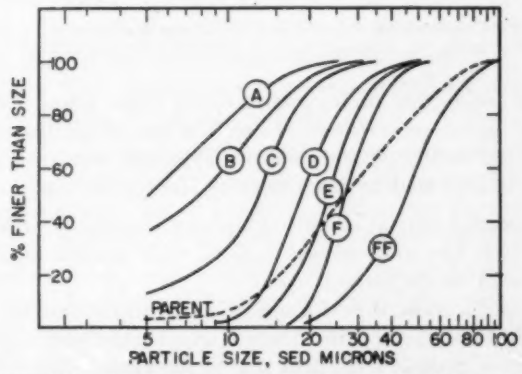


Fig. 8. Cumulative size distribution curves of the parent flour and its seven subsieve-size fractions.

Though the visual concept of Fig. 7 serves to clarify the size properties of the fractions, the cumulative size curves have been found to be more convenient in practice. The latter are useful in the graphical determination of critical cut which, in turn, defines the flowdynamic size ranges of specific fractions between two classification steps (Fig. 8).

The protein contents of the coarse and fine fractions and the critical cut values obtained from the cumulative percent finer-than-size curves, as illustrated in Fig. 2, have practical application in determining the character of the protein shifting behavior of a flour. The latter is obtained by plotting the protein content of the fractions of A and AA as a function of the critical cut size; the protein content of the blend of A plus B calculated from the protein content and yield of the individual fractions and the protein content of BB as a function of the critical cut size at process step (b). Thus, the cumulative protein content of the fines, when plotted against critical cut size, has a negative slope as illustrated in Fig. 9.

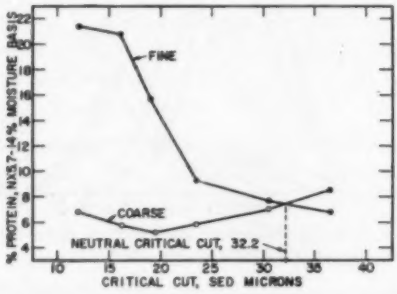


Fig. 9. Protein contents of the fine and coarse fractions plotted as functions of critical cut, illustrating the intercept formed called the neutral critical cut.

The two converging curves intersect at a point whose abscissa has been named the *neutral critical cut* size. The size of the neutral critical cut in this case is 32.2 SED microns. The significance of the neutral critical cut is indicated by the following interpretations:

1. The neutral critical cut size designates that size at which a classification yields fine and coarse fractions with protein equal to the protein content of the parent stock.
2. Size classification, if performed at a critical cut smaller than the neutral critical cut, yields a fine fraction with higher, and a coarse fraction with lower, protein level than that of the parent flour.
3. Size classification, if performed at a critical cut larger than the

neutral critical cut, yields a fine fraction with lower, and a coarse fraction with higher protein level than that of the parent flour.

The neutral critical cut, therefore, designates a narrow particle size region below which elementary flour particles exist, which inherently possess properties such as size, shape, and density that are contributive to protein shifting. Above the particle size of the neutral critical cut, no dramatic protein shifting in the endosperm chunk particles can be expected.

Other Analytical Indices. A number of analytical indices of the seven SSS fractions and their parent flours can be found in Tables II and III. Though the protein shifting is the most important property change in the fractions, other chemical and physical property changes are listed and discussed here.

The Fisher average particle size increases with the particle size ranges, as would be expected. The Fisher sizes indicate most likely some measure of specific surface; with decreasing Fisher value the specific surface increases (5,8,9). The moisture content is lower in the smaller particle size ranges. This would be the expected result of air-handling, because of the higher specific surface of the small particles. The ash content of the fractions varies, being generally higher in the finer fractions; i.e., as protein content increases the ash content also increases. The distribution of maltose values appears to be highly significant and, as may have been anticipated, follows the concentration of available starch substrate and/or the enzymatic concentration; the latter generally proportional to protein content. The alkaline water retention capacity of the fractions appears to follow primarily the specific surface and/or the protein content and perhaps other unknown components.

Though pH values vary only between 5.50 and 5.83, there is a definite trend for lower pH values to be associated with higher protein content. The petroleum ether-extractable lipid content of the fractions increases with protein content.

The specific gravity of the fractions confirms the microscopic observations as the depletion of protein results in an increase of specific gravity approaching that of starch. Conversely, an increase of protein in a fraction decreases the specific gravity of the material.

Thiamine content is generally higher in the high-protein fractions, but some loss is indicated, because the cumulative thiamine content of the fractions is less than the thiamine content of the parent flour. This discrepancy may be due to the precision of the method at the low thiamine level in some of the fractions.

TABLE II

CHARACTERISTIC INDICES OF SOFT WHEAT FLOUR AND ITS SEVEN SUBSIEVE-SIZE FRACTIONS OBTAINED BY THE USE OF A FLOWDYNAMIC PRINCIPLE BY AIR CLASSIFICATION AT INCREASING CRITICAL CUT SIZE

	Yield as Percent of Parent	Critical Cut Size Range	Sharpness	Average Particle Size	Protein [a] (N × 5.7)	Moisture	Ash	Diastatic Activity	AWRC [b]	pH	Specific Gravity	Bulk Density	Color Reflectance Rd by Hunter	Vitamin B_1 Thiamine	Viscosity	Fat by Petroleum Ether Extraction
		SED μ	%	*Fisher* μ	%	%	%	*mg maltose/ 10 g*	%		*g/ml*	*kg/l*		*mg/lb*	*°MacM*	%
Parent	100.0	min-max	..	12.0	7.8	11.7	0.30	88	57.7	5.80	1.455	0.507	65.2	0.17	74	0.74
A	5.0	min–12	78	3.8	22.2	8.2	0.38	155	88.8	5.50	1.417	0.266	64.3	0.13	300+	1.78
B	7.0	+12 –16.2	76	4.3	20.0	9.1	0.37	163	97.1	5.51	1.403	0.287	64.4	0.22	300+	1.56
C	10.4	+16.2–19.0	72	8.1	10.0	9.5	0.33	143	78.5	5.62	1.443	0.400	66.1	0.09	183	0.87
D	22.0	+19.0–23.5	50	14.4	3.1	11.0	0.30	89	60.3	5.82	1.472	0.578	72.0	0.04	3	0.39
E	15.0	+23.5–30.5	43	16.4	2.5	10.8	0.28	76	58.4	5.83	1.472	0.647	69.4	0.04	3	0.33
F	12.4	30.5–36.5	36	16.5	3.3	10.7	0.31	72	55.4	5.82	1.487	0.681	59.1	0.04	5	0.40
FF	28.2	36.5–max	..	23.0	8.6	10.5	0.32	63	45.9	5.70	1.461	0.646	69.5	0.09	58	0.82
AA	95.0	+12 –max		13.4	6.8	11.3	0.32	101	48.4							
BB	88.0	+16.2–max		15.3	5.7	11.0	0.30	99	51.9							
CC	77.6	+19.0–max		18.0	5.2	10.6	0.31	78	49.5							
DD	55.6	+23.5–max		17.9	5.8	11.0	0.32	63	48.5							
EE	40.6	+30.5–max		19.9	7.0	10.8	0.31	66	44.5							

[a] Protein and other test results are expressed on a 14% moisture basis.
[b] Alkaline water retention capacity.

TABLE III
MAJOR INDICES FROM PHYSICAL TESTING METHODS OF A SOFT WHEAT FLOUR AND ITS SEVEN SUBSIEVE-SIZE FRACTIONS

Product	Absorption	Farinograph					Extensigraph			Amylograph
		A[a]	B[a]	C[a]	D[a]	Valorimeter	Extensibility	Resistance	Area	Peak Viscosity
		Dimension								
							mm	*Bu*	cm^2	*Au*
Parent	53.7	0.4	0.9	1.2	65	40	149	200	48.0	690
A	78.6	22.2	29.9	19.6	25	100+	268	160	120.0	445
B	79.6	19.1	34.4	28.2	15	100+	263	160	114.5	470
C	61.5	1.2	2.6	16.8	30	57	192	110	43.0	580
D	57.2	0.4	0.5	0.2	195	16	61	...	16.0	760
E	54.3	0.5	0.6	0.3	205	18	22	...	9.0	800
F	53.5	0.4	0.6	0.3	195	17	68	...	17.5	795
FF	54.2	0.6	1.7	8.1	25	47	183	150	107.5	685

[a] A, time in minutes from the start of water addition until the top of the curve intercepts the 500 B.u. line, "arrival time." B, C, and D, respectively, equivalent to Dough Development Time, Stability, and Tolerance Index, *Cereal Laboratory Methods*, 6th ed., 26.4.

The bulk density data show a general increase with protein depletion as a result of decreasing specific surface.

The results of the farinograph, extensigraph, and amylograph tests are listed in Table III. The pronounced mixing, extension, and gelatinization properties are evident from the table and Figs. 10, 11, and 12. Dough development times increase with protein content. Absorptions, though high for the protein fractions, do not appear to relate directly to the protein content when A and B fractions are compared. At the other extreme, fractions D, E, and F, the low-protein fractions, have low absorption, though this is not necessarily related to protein content. The general shape characteristics of the curves do reveal

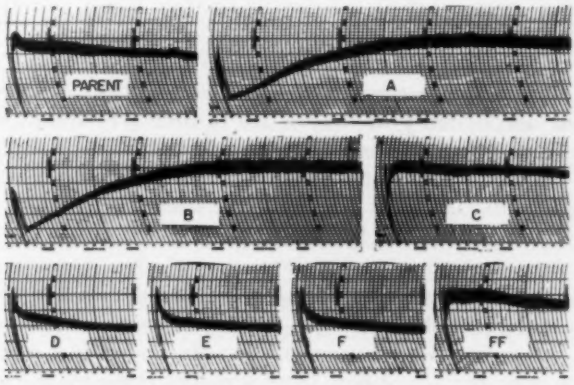


Fig. 10. Farinograph curve characteristics of the parent and the seven subsieve-size fractions.

property changes associated with protein content, such as dough development time and stability (Fig. 10).

The extensigraph results illustrate the changes in dough handling properties of the various fractions. Though there are changes in extensibility and resistance which are interesting in themselves, the major changes can be followed by the area under the 1-hour curve which changes tenfold or more between the lowest and the highest protein fractions (Fig. 11). The greatest area under the curve is found in the A fraction. The area then decreases progressively to a minimum in fraction E and then increases through fractions F and FF.

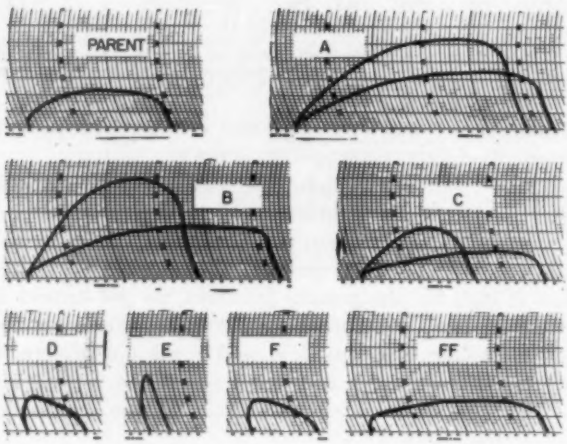


Fig. 11. Extensigraph curves of the parent flour and the seven subsieve-size fractions.

The amylograph results confirm the maltose values to the extent that lower amylogram peak heights are exhibited by the higher protein fractions. The data would indicate either a concentration of the amylase enzymes, or a substrate more susceptible to enzymatic attack, or perhaps both. The peak height would also be lowered by the depletion of starch in the high protein fractions (Fig. 12).

The Factors of Flour Particle Flowdynamic Size. Calculations for the flowdynamic relations of particles, such as terminal velocity, viscous resistance, and other concepts, involve three important variables; namely, the geometric measurement, density and shape (6,12). The following is an analysis of how these variables influence the measurement of flowdynamically sized flour particles.

The heterogeneous structure of flour endosperm presents some problems for the evaluation of density. If, however, the problem is considered from the aspect of density differences which exist between the principal components of endosperm flour, the protein matter, and the starch granules, it can be demonstrated that the effect of density variations is small in the postulated flowdynamic size figure.

If Stokes' law is applied and 1.49 and 1.32 g. per ml. are used as the approximate density values for starch granules and protein matter, respectively, then an imaginary spherical particle of protein matter has to have a diameter 1.17 times larger than the imaginary spherical

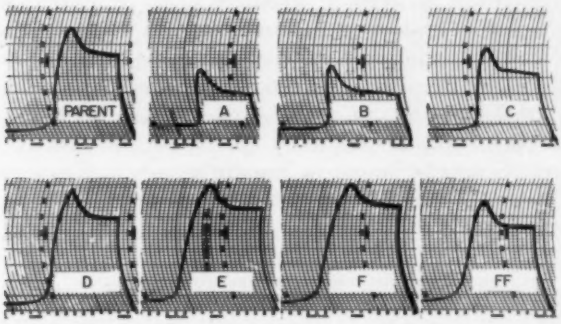


Fig. 12. Amylograph curves of the parent flour and the seven subsieve-size fractions.

starch granule to be measured as equivalent based on common settling velocity in the viscous medium of kerosene. In the less viscous medium of air, this factor is reduced to 1.06. To illustrate, this would mean that a 10-micron sphere of starch and an 11.7-micron spherical protein particle would be identically sized by flowdynamic methods in benzene. With the average density of 1.44 for flour particles, the 10-micron spherical starch particle would be measured to have 10.4 SED micron size, and a 10-micron spherical protein particle would be measured as 8.9 SED micron size in kerosene.

Since flour particles do not have ideal geometric configurations, the values obtained by flowdynamic measurements have to rely on postulations. In flowdynamic relations Stokes Effective Diameter (SED) is a convenient postulation; particles which have a common settling velocity are considered to be the same size, irrespective of values obtained by other measurements. In the specific case of flour, each particle is attributed a postulative size, i.e., the diameter of a sphere of

1.44 density having common settling velocity with the particle it defines.

It is evident from the photomicrographs and the discussion that the value applied to a protein particle and starch particle, by virtue of the flowdynamic or sedimentation postulation, may be quite different from that obtained by direct observation. This discrepancy sparked some investigation to compare the shape character of protein particles with that of starch particles at arbitrary flowdynamic sizes; that is, some shape factors were wanted.

Comparison of geometrical measurements on microscopic slides made of arbitrary sections of a sedimentation column (in the present case the column in the Whitby centrifuge tube) permitted calculation of shape factors. These shape factors use direct and calculated measurements and elucidate the differences occurring between the shapes of protein matter particles and of starch granules, both of which were measured by the flowdynamic method into the same arbitrary size category. The calculations are limited to one- or two-dimensional concepts and, therefore, may be poorer in descriptive power than three-dimensional calculations. The resulting relationship warranted the judgment

TABLE IV
SHAPE FACTORS CHARACTERIZING PROTEIN MATTER PARTICLES AND STARCH GRANULES

Sedimentation Column	Φ protein	ϕ protein	ϕ starch	$\frac{\Phi \text{ protein}}{\phi \text{ protein}}$	$\frac{\Phi \text{ protein}}{\phi \text{ starch}}$	$\frac{A_\phi \text{ protein}}{A_\phi \text{ starch}}$	$\frac{A_\Phi \text{ protein}}{A_\phi \text{ protein}}$	$\frac{A_\Phi \text{ protein}}{A_\phi \text{ starch}}$
	a	b	c	d	e	f	g	h
mm	μ	μ	μ					
0–1	21.0	10.6	11.1	1.98	1.89	0.91	3.49	3.18
2–3	19.0	12.9	9.5	1.47	2.00	1.84	2.17	4.00
5–6	17.1	11.4	9.5	1.50	1.80	1.45	2.25	3.24
10–11	14.0	7.0	6.0	2.0	2.34	1.36	4.00	5.44
Shape factor averages				1.74	2.00	1.39	2.98	3.96

a) Φ protein = the maximum linear measurement of the projected image of protein matter particle.
b) ϕ protein = diameter of a circle which is equivalent to the projected area of the protein matter particle.
c) ϕ starch = the largest diameter of the projected image of starch granule.
d) Φ protein/ϕprotein = ratio of the maximum linear measurement of the projected image of protein matter particle to the diameter of circle which is equivalent to the projected area of the protein matter particle.
e) Φ protein/ϕstarch = ratio of the maximum linear measurement of the projected image of protein matter particle to the largest diameter of the projected image of starch granule.
f) A_ϕ protein/$A_\phi$starch = ratio of the projected area of protein matter particle to the projected area of starch granule (see ϕ starch).
g) $A\Phi$ protein/A_ϕ protein = ratio of circle area covering image of protein matter particle (see Φ protein) to projected area of protein matter particle.
h) $A\Phi$ protein/A_ϕ starch = ratio of circle area covering image of protein matter particle (see Φ protein) to the projected area of starch granule (see ϕ starch).

that these dimensions would be satisfactory for explanation of the phenomena.

Table IV records the relationships which were calculated from the results of microscopic measurements of the maximum-size protein matter particles and maximum-size starch granules on slides from four arbitrary sections at four different flowdynamic sizes of the sedimentation column.

Following is an explanation of the symbols used:

φ protein equals the diameter of a circle whose area is equivalent to the projected area of the protein matter particle at the position of maximum stability – calculated.

ϕ starch equals the largest diameter of a projected image of starch granule nearly spherical at the position of maximum stability – actually measured. The image is a close approximation of a circle.

Φ protein equals the maximum linear measurement of the projected image of protein matter particle – actually measured.

A ϕ protein equals the projected area of the protein matter particle – actually measured. This area is equal to the area of a circle with ϕ protein diameter.

A ϕ starch equals the area of a circle with ϕ starch diameter – calculated.

A Φ protein equals the area of a circle which has Φ diameter – calculated.

The Φ protein/ϕ protein and A Φ protein/A ϕ protein factors express numerically a degree of irregularity of the protein matter particles using linear and areal concepts, respectively. The Φ protein/ϕ starch, A Φ protein/A ϕ starch, and A ϕ protein/A ϕ starch factors express numerically how many times the protein matter particles are larger than the flowdynamically identical-sized starch granules, using other than the SED postulation.

According to Herdan (12), the shape factors are generalized into three groups: 1) they are factors of proportionality between particle size determination results by different methods; 2) they are the conversion factors for expressing determination results in terms of an "equivalent" sphere; and 3) they transform the second and third power of the variable, particle diameter, into the particle surface and particle volume, respectively.

The irregularity factors of protein matter particles, namely, Φ protein/ϕ protein and A Φ protein/A ϕ protein, may belong in Herdan's second group, in that they correlate actual microscopic measurements on the same particle with calculated values. However, the comparison of shape character of protein particles and starch granules, such

as is expressed by Φ protein/ϕ starch, $A\Phi$ protein/$A\phi$ starch, $A\phi$ protein/$A\phi$ starch factors, do not belong in Herdan's shape factor groups but may be called *relative shape factors.*

If it is considered that velocity and resistance, the basic factors in flowdynamic sizing, are proportional to the particle diameter, and at Reynolds number larger than 2 (6) they are proportional to the particle diameter having an exponent larger than 1, the relative shape factor values between the linear and areal extremes – namely, between 2.0 and 3.96 – may well express how much times more irregular-shaped protein matter particles have than starch granules.

This relative irregularity factor may be corrected by the specific gravity proportions $2/1.17 = 1.71 \sim 3.96/1.17 = 3.39$, arriving at an approximation of the effect of shape alone on the irregularity as compared to our case with the Whitby sedimentation tube, where the effect of specific gravity and shape were combined.

The presented discussion shows that different flour particles, especially in the subsieve-size range, contain different proportions of protein and starch, and that they differ also in flowdynamic characteristics, especially by their geometric measurement, density, and shape. The application of flowdynamic principles in size grading takes advantage of these differences and makes it possible to concentrate protein matter and/or starch granules in specific size ranges.

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DEVELOPMENT OF THE LEAVENING SYSTEM FOR AN INSTANT BREAD MIX[1]

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ABSTRACT

A leavening system consisting of 3.5% sodium bicarbonate and 9% glucono-delta-lactone (flour basis) has been developed for a bread mix which requires only 10 minutes to prepare from the time of addition of water to insertion of dough into the oven. The baked item produced from this mix closely resembles yeast-leavened bread in grain, texture, volume, and appearance, but not in flavor. Variations in sodium bicarbonate, acid, and total leavening indicated optimum performance at approximately 3.5% sodium bicarbonate, 9% acid, and between 12 and 15% total leavening based on the flour weight. A rather remarkable tolerance to changes in dough temperature from 55°F. (12.8°C.) to 85°F. (29.4°C.) and proof time from 5 to 60 minutes was indicated. There was a wide range in mixing tolerance between 2 and 10 minutes, although a considerable decline in loaf volume occurred as a result of prolonging the mixing time beyond 10 minutes.

The Armed Forces require a complete bread mix which can be prepared with a minimum of equipment and skill and which will re-

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main acceptable for long periods when stored under adverse conditions. Since yeast is thermally unstable and is very sensitive to changes in environmental conditions during fermentation, it was desired to exclude this ingredient from the formulation. In conventional baking processes, large volumes of dough must be maintained at controlled temperatures and humidities, the doughs and ingredients are very sensitive to variations in handling, and the processing of the doughs takes an inordinately long time. Use of broth methods does not ameliorate these difficulties very much and adds some further problems, such as that of holding a large tank of broth at a controlled temperature for several hours.

It is desired that bread be made in a simplified field bakery in a small continuous mixer. In atomic warfare where combat forces will consist of highly dispersed units of approximately 2,000 men, the current mobile or portable bakeries are inefficient. A simplified field baking system would offer savings in time and equipment, and would simplify problems in logistics.

These considerations motivated the attempt to develop a process which would circumvent the fermentation steps in bread-baking. This paper describes the results achieved in one phase of that project.

Materials and Methods

Materials. The flour used was a good commercial grade of hard wheat flour, Baker's Patent, analyzing 11.9% protein and 0.47% ash on 14% moisture basis. The yeast used was compressed baker's yeast never more than 7 days old and held at 38°F. (3.3°C.) prior to use. Shortening was 100-hour vegetable shortening. The glucono-delta-lactone (GDL) was a mixture of 95% GDL and 5% calcium stearate. A large number of organic and inorganic acids were tested. For the most part the organic acids tested were various mono-, di-, and tricarboxylic acids. The inorganic acids tested were common leavening acids.

Methods. The formula for standard Army bread was followed as closely as possible in designing the instant bread formula to be used with chemical leavening agents. The "control" for the experiments on instant bread comprised bread made by the standard formula given below and prepared according to the directions following.

Standard Army Bread Formulation

	% flour weight
Flour	100.0
Sucrose	5.5
Nonfat dry milk	6.0
Salt	1.0
Shortening	5.0
Compressed yeast	2.0
Water	65.0

The yeast was dissolved in a portion of the absorption water and placed in the McDuffee bowl on a Hobart A-200 mixer, with all the remaining ingredients. The dough was mixed for 1 minute at slow speed and 7 minutes at second speed. Dough pieces were scaled at 20½ oz. into individual fermentation jars, allowed to ferment for 2 hours, then punched and given a 20-minute rest before molding. The loaves were proofed 60 minutes at 100°F. (37.8°C.) and 90% relative humidity (r.h.), and baked for 30 minutes at 425°F. (218.3°C.) in a gas-fired reel oven.

The formula for the instant bread mix is shown below.

Instant Bread Mix Formulation

	Percent flour weight	g
Flour	100.0	700.0
Sucrose	5.5	38.5
Nonfat dry milk	6.0	42.0
Salt	1.0	7.0
Shortening	5.0	35.0
Soda	3.5	24.5
Delta lactone of gluconic acid	9.0	63.0
	130.0	910.0

In making instant bread, the dry mix (910 g.) and 385 ml. water (55% of flour weight) were mixed for 1 minute at first speed in a McDuffee bowl on a Hobart A-200 mixer, and then mixed for 7 minutes at second speed. The dough was manually scraped from the sides of the mixing bowl several times during the mixing stage. After mixing, the dough was immediately scaled at 20 oz., rounded, molded, panned, and then given 5 minutes' rest time at 100°F. (37.8°C.) and 90% r.h. Room temperature of about 75°F. (23.9°C.) was considered satisfactory for proofing. The loaves were baked at 425°F. (218.3°C.) until the crust color indicated that they were done, or about 30 minutes.

In testing the organic and inorganic acids, sodium bicarbonate was utilized as the source of leavening gas at a level of 35% of the flour weight. The pH of the baked Army bread was the criterion employed in determining the proper level of acid to be used. In judging

the adequacy of chemicals for leavening purposes, much use was made of a subjective evaluation of gassing behavior of doughs. When a dough possessed the ability to proof satisfactorily to the proper height without undergoing tearing or other undesirable physical changes, it was then carried through to the finished loaf stage. The baked loaf volume was considered a good indicator of the behavior of leavening agents. Vigorous gassing in the mixing bowl was taken as an indication of poor utility, and such doughs were rarely baked. The taste and appearance of bread was judged by three or four persons familiar with baking procedures. Numerical scores were usually not assigned.

Hydrogen peroxide with catalase, or with yeast as a source of catalase, was examined for possible use as a leavening system (2,5,6). The levels of hydrogen peroxide and yeast tested were those of Rotsch (5).

Volume determinations were made by the usual rapeseed displacement method. pH was determined electrometrically on mixtures of 10 g. of crustless crumb in 100 g. distilled water, 10 minutes after mixing.

The level of sodium bicarbonate was varied from 2 to 5% of the flour weight, with all other conditions held constant. The acid level was varied from 7 to 15% of the flour weight. The total leavening was varied, keeping the ratio of acid to sodium bicarbonate constant from 5 to 20% of the flour weight. Mixing time in second speed was varied from 1 to 15 minutes. Proof time was varied from 5 to 60 minutes. Dough temperatures were varied from approximately 55° to 130°F. (12.8°–54.5°C.).

Results and Discussion

Leavening Systems Other than Acid and Sodium Bicarbonate. Carbonic anhydrase was included in a bread formula with ammonium carbonate. Little leavening action was observed. However, a pervading odor of ammonia eliminated this material from consideration. Sodium carbonate was tested with GDL and appeared to offer no advantage over sodium bicarbonate at the same level.

Baked loaves made with peroxide-catalase were of desirable volume and had extremely fine grain. The crust appearance of the baked loaf was not typical of yeast-leavened bread, and the crumb texture was extremely doughy. This condition is mentioned in a patent, which asserts that if the peroxide has not fallen below a certain percentage by the time the dough is baked, the loaf will come out of the oven, after the usual baking period, with a doughy, unleavened texture (4).

Leavening Systems Employing Various Acids. The next part of the investigation was devoted to a study of the utility of various acids, organic and inorganic, in combination with sodium bicarbonate. The pH of the Army bread was used as an indication of the pH to be obtained with acid and sodium bicarbonate. Of the inorganic acids used, monocalcium phosphate, both the anhydrous and the monohydrate used individually, sodium aluminum sulfate, anhydrous sodium aluminum phosphate, monobasic sodium phosphate, and dicalcium phosphate were rejected for causing too rapid gassing. Sodium acid pyrophosphate gave a controlled leavening reaction, but was rejected on the basis of an off-taste contributed to the bread when used at levels necessary in the instant mix. Hydrated sodium aluminum phosphate is a controlled leavening acid which may show promise.

A large group of organic acids were tested for adequacy. These were for the most part various mono-, di-, and tricarboxylic acids. The first group were grossly inadequate from the standpoint of gassing performance:

cream of tartar	lactic	malic
fumaric	aconitic	succinic
tartaric	glutaric	malonic
mucic	itaconic	mandelic
citric	quinic	mesaconic
acetic	glycolic	citraconic

The second group includes those acids contributing off-flavors or off-odors to the finished bread: cinnamic, maleic, heptanoic, nonanoic, and sebacic.

The third group includes acids selected for further examination: aluminum formate, glucono-delta-lactone, adipic and azeleic acids.

Adipic, azeleic, and sebacic acids may possibly warrant further investigation. These aliphatic dicarboxylic acids have received only limited examination. Cinnamic acid showed some promise from the controlled leavening aspect, but gave a yellow crumb color which was due to the color of the acid crystals (possibly impurities). The pH of the baked loaf utilizing cinnamic acid was 5.8. Loaves exhibiting yellow crumb color due to high alkalinity usually had a pH above 8.0. In a few instances, an acid of one group also possessed the attributes of another group such as gassing in the mixing bowl and causing an off-odor or flavor. Heptanoic and nonanoic acids are two examples. In addition, these two acids are liquids and would possess certain disadvantages when used in a prepared mix. Cinnamic and sebacic acids

possess the advantage of a controlled leavening acid but also have the disadvantage of having an odor not typical of bread.

On the basis of these preliminary investigations, the delta lactone of gluconic acid was selected as the most promising acid. Aluminum formate and the hydrate of sodium aluminum phosphate showed some utility and may be worthy of further examination. The possibility of a toxicity problem involved in the use of formate resulted in elimination of further study with this compound. Later studies were confined to the delta lactone of gluconic acid.

Glucono-Delta-Lactone. The delta lactone of gluconic acid is prepared by controlled oxidation of D-glucose by a bacterium. The Committee on Foods of the American Medical Association has approved the product for inclusion in the list of accepted foods (1). Toxicity of glucono-delta-lactone has been studied by Sisk and Toenhart (7), who used gluconic acid therapeutically as a urinary acidifying agent. They found that it was well tolerated by humans and was eliminated unchanged in the urine. Hermann (3) also used gluconic acid to dissolve urinary calculi and found that it caused no side reactions. In its lactone form the compound is neutral, but under certain conditions it hydrolyzes fairly rapidly to give the free acid which can then react with sodium bicarbonate. No doubt it is this property which governs the rate of reaction of the compound in leavening systems.

Since there is presumably not a direct, rapid, mole-for-mole reaction between the acid and sodium bicarbonate, a study was made of the effect of varying the acid to sodium bicarbonate ratio on loaf characteristics.

The volumes and pH values are shown in Fig. 1, where the sodium bicarbonate was varied and the acid weight was held constant at 9% of the flour weight. The loaf volumes reached a maximum at approximately the 3.5% sodium bicarbonate level. Percentages for all the ingredients were calculated on the weight of flour.

At the highest sodium bicarbonate level the dough when removed from the mixing bowl felt "short," like biscuit dough, and the crumb of the baked loaf was yellow. Ability of the dough to form a skin was absent. The loaves had a shell-crust or tendency to wild break-and-shred. As the sodium bicarbonate was increased, there was an appearance of "soda spots" on the top surface of the crust.

Figure 2 shows the volumes and pH values of the baked loaves when the lactone weight was varied and the sodium bicarbonate was held constant at the 3.5% level. Loaf volumes increased slightly with increasing lactone.

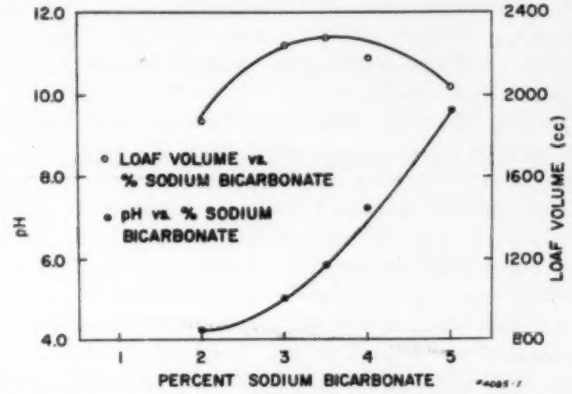

Fig. 1. Effect on pH and loaf volume with sodium bicarbonate variable and acid constant (9.0% of flour weight).

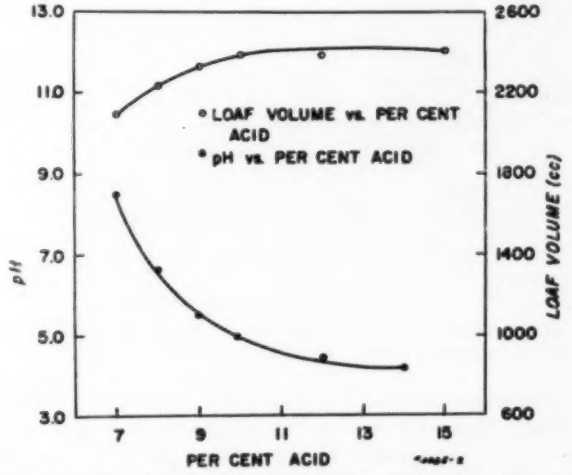

Fig. 2. Effect on pH and loaf volume with acid variable and sodium bicarbonate constant (3.5% of flour weight).

There were some soda spots on the top surface of the crust at the lower levels of GDL. These soda spots became less noticeable in the baked loaves as the lactone weight was increased. No spotting was observed at the 12 and 15% levels, but the taste was very sour. The taste of the bread made with 10% GDL was, at times, noticeably different from that of ordinary bread. Nine percent GDL was about optimum

for loaf volume and gave a barely perceptible acid taste. Consequently, this level was accepted as a base for further formulation work, even though wild break-and-shred and a tendency toward shell-tops were observed in the baked loaves.

The data in this paper were based on a one-stage complete mix. Earlier work with a two-stage mixing procedure, where either the acid or soda was mixed with the shortening and added near the completion of the dough-mixing, produced loaves with volumes approximately 10% greater than those with the one-stage mixing. The appearance of the baked loaves from the two-stage mixing method was better, with less tendency toward shell-tops and erratic break-and-shred.

As a result of these experiments, a ratio of 9% of the GDL-calcium stearate mixture to 3.5% of sodium bicarbonate based on the flour weight was used in subsequent work. This corresponds to a molecular weight ratio of 1.21 of acid to 1 of sodium bicarbonate.

The effect of varying total leavening ingredients on loaf volume is shown in Fig. 3.

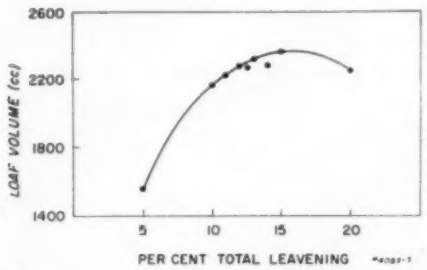

Fig. 3. Effect on loaf volume of varying total leavening, acid: sodium bicarbonate ratio constant (9 parts acid: 3.5 parts sodium bicarbonate).

This experiment showed that maximum volume was reached when approximately 12.5% of total leavening agents was employed. Further studies used this percentage. The dough at the 20% total leavening level was short and had handling properties somewhat like those of a biscuit dough. Spotting of the top surface of the crust was evident. At intermediate leavening levels the appearance of soda spots was slight. There was again a tendency to shelling and wild break-and-shred. The appearance of soda spots was found to be corrected by extra mixing, decreasing the sodium bicarbonate, increasing acid, and decreasing the particle size of the sodium bicarbonate.

Next, the temperature of the dough was varied by using circulating water at the selected temperature through the jacket of the McDuffee

bowl and water of the desired temperature in the dough. Results are shown in Fig. 4.

These data indicate a rather remarkable tolerance to changes in dough temperature. This is a highly desirable characteristic for mixes destined for field use. Spotting was quite pronounced on the baked

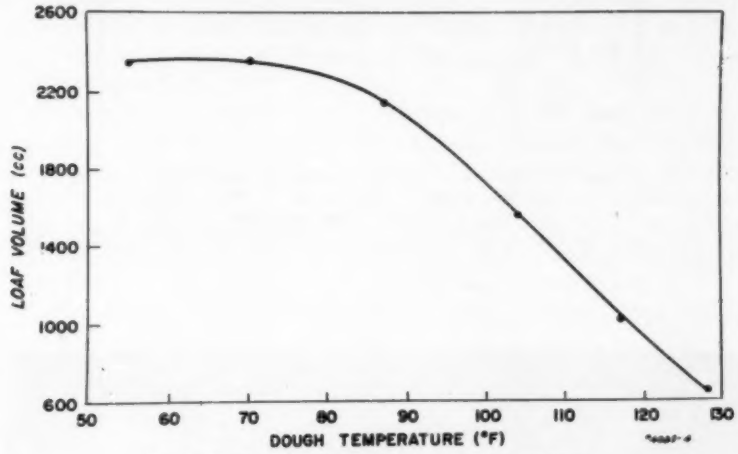

Fig. 4. Effect of dough temperature on loaf volume.

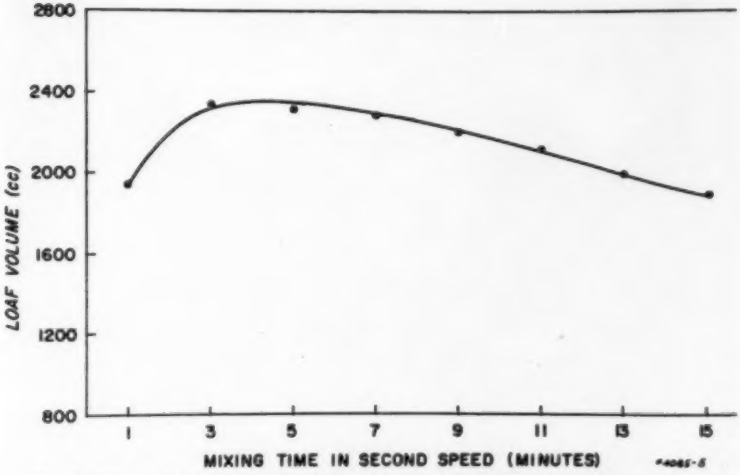

Fig. 5. Effect of mixing and molding time on loaf volume, all other conditions held constant.

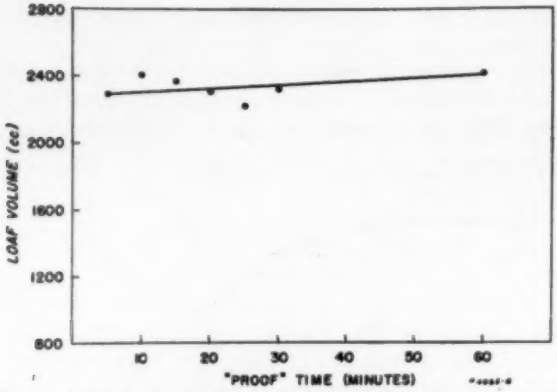

Fig. 6. Effect of varying proof time on loaf volume, all other conditions held constant.

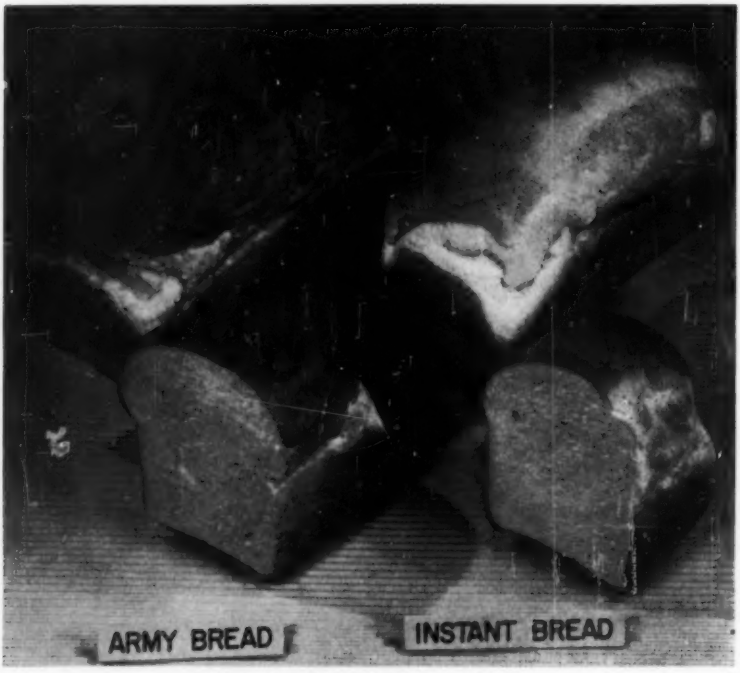

Fig. 7. Army bread vs. instant bread.

loaves from the 55°F. (12.8°C.) dough temperature. Much gas was lost from the doughs having dough temperatures of 104°, 117°, and 128°F. (40°, 47.2°, and 53.3°C.). The baked loaves were again shell-topped.

An experiment was performed in which the mixing and molding time was varied over a considerable range, with all other conditions held constant. Temperatures of the doughs varied between 70° and 79°F. (21.1° and 26.1°C.). Results are shown in Fig. 5. There was a considerable decline in volume as a result of prolonging the mixing and molding time.

The proof time, or the time the molded dough must rest in the pan before it is placed in the oven, should be short for field use. For experimental purposes, the molding procedure is considered as a continuation of the mixing process, since appreciable gas retention does not begin until the dough emerges from the molder. Some gas is lost in the mixing and molding steps. Figure 6 shows the results of varying the proof time. Again a highly desirable tolerance is apparent.

Figure 7 shows a loaf of the baked instant bread with a loaf of yeast-leavened Army bread for comparison.

Comparative physical and chemical data on the two kinds of bread are given below.

	Army Bread	*Instant Bread*
Volume	2,800	2,300
pH	5.85	5.5
Grain	open	fine, thin-walled
Texture	good	good
Color of crust	dark brown	tan
Crust appearance	good	slight spotting
Symmetry	good	shell-topping
Taste	good	bland

Bread baked with this formulation and procedure has been found to be 1) equal in physical characteristics to yeast-leavened bread and capable of being prepared without the intervention of a fermentation step; 2) bland in taste, having no objectionable odor or flavor, but also not having the odor or flavor of yeast-leavened bread.

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EFFECTS OF THE WATER-SOLUBLE CONSTITUENTS OF WHEAT FLOUR ON COOKIE DIAMETER[1]

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ABSTRACT

The fraction of wheat flour water-solubles that contained the low-molecular substances had negligible effects on cookie diameter, although this fraction contained 68–78% of the original water-solubles, most of the hexose material, and much of the nitrogenous matter, but very little of the pentose material. The water-soluble proteins from the high-molecular fraction had small effects on cookie diameter. The water-soluble polysaccharides from the high-molecular fraction greatly reduced cookie diameter. These polysaccharides contained 40–70% pentoses and comprised 60–70% of the high-molecular fraction of the water-solubles. Most of the reduction of cookie diameter caused by the water-solubles was due to the polysaccharides with high pentose content.

A wheat variety is considered to have good soft-wheat quality (3) when the flour from it yields a cookie of large diameter with the top surface broken by fairly wide cracks somewhat evenly spaced to give uniformly sized islands. Most quality factors for cookie baking, such as protein, are inversely related to cookie diameter. This is opposite to bread-baking in which the quality factors, such as protein, are directly related to loaf volume (6).

Yamazaki found that purified tailings had a large, deleterious effect on cookie diameter (25). He concluded that any component capable of absorbing large amounts of water would affect cookie diameter. Sollars (21) showed that the tailings fraction exerted a large effect and that the water-soluble fraction had less effect on cookie diameter. However, of the four major fractions, the water-soluble fraction had the largest diameter effect per gram of material (21).

Inasmuch as the tailings fraction is difficult to fractionate, the water-soluble fraction was chosen for investigation. Several reports give methods for separating the water-soluble fraction (2,8,16,17,19,20). This paper reports the separation of the principal constituents of the water-soluble fraction and the determination of their influence on cookie diameter. The pentosans were expected to have a part in the diameter effect because these substances absorb relatively large

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amounts of water (2,23). The soluble proteins are important in bread-baking (17,18) and were included in this study.

Materials and General Methods

Straight-grade, unbleached flours of 7–8% protein content were Buhler-milled from pure variety composites. Rio is a hard red winter wheat variety with poor pastry quality and good bread-baking quality. Elgin and Elmar are soft white club wheat varieties with good pastry quality. Marfed, Golden, and Brevor are soft white common wheat varieties with good pastry quality. This study was begun with Elgin and Rio (Rio 53) flours. Near the end of the study these flours were exhausted, and Elmar and another Rio flour (Rio 57) were used in their place.

Preparation of Water-Extracts. One part of flour was added to 3 parts of distilled water. After preliminary mixing, the suspension was allowed to stand for 10 minutes, stirred for 10 minutes, and centrifuged for 10 minutes. The supernatant was filtered through glass wool. This extract was then treated as described later or concentrated under vacuum below 30°C., shelled, and lyophilized (25) to obtain the total water-solubles. The extract was not heated unless indicated.

Fractionation of Water-Solubles by Alcohol. Frequently the alcohol-insoluble material (crude pentosans) was prepared from the water-extract by the procedure of Perlin (19), and, in addition, the alcohol-soluble material was recovered. The water-extract was poured into 3 volumes of 95% ethanol, and the mixture was stirred vigorously. The white precipitate was recovered by centrifuging, redissolved in water, shelled, and lyophilized. The supernatant (material soluble in 71% ethanol) was concentrated under vacuum below 30°C. to a thick syrup, water was added, and the solution was again concentrated to a thick syrup. The syrup was diluted with water, shelled, and lyophilized.

Cookie-Baking Method. The micro baking procedure of Finney, Morris, and Yamazaki (7) was used. The average diameter of two cookies baked from a 40.0 g. sample of a standard cookie flour was determined. For 52 such samples from the standard flour baked on 18 different days over a period of 2 years, the average cookie diameter was 8.95 cm., the standard deviation was 0.10 cm. or 1.0 mm., and the least significant difference was 0.04 cm., or 0.4 mm.

The effect of the fractions on cookie diameter was determined by adding 1.0 g. of the fraction to the 40.0 g. sample of the standard flour (25) and recording the changes in cookie diameter in millimeters as compared with the diameter of cookies from the standard flour alone. As more potent substances were obtained, fractions of a gram were

added to the standard flour. Assuming the same variation in diameter of the cookies from these samples with the added fractions, as in the control for which there was a sample size of 52, the average least significant difference at the 5% level for the samples with added fractions would be 0.18 cm., or 1.8 mm.

Analytical Methods. Protein and moisture analyses were made by conventional methods (1,11). A factor of 6.25 was used for conversion of nitrogen to protein (8).

Pentosans were determined by a method modified from that of Wolf *et al.* (24). Samples containing 5–30 mg. of pentoses were added to 50 ml. of 12% hydrochloric acid in typical pentosan distillation units. Additional 12% hydrochloric acid was added during distillation, and 400 ml. of distillate were collected. The distillate was made up to a 500 ml. volume with 12% hydrochloric acid, and a 100-ml. aliquot was redistilled as suggested by Kullgren and Tyden (12). During redistillation, 50 ml. of 12% hydrochloric acid were added, and 100 ml. distillate were collected.

Furfural in the final distillate was determined by the bromination method of Hughes and Acree (9). The reaction time was 4 minutes at 0°C. ± 1°C. Sodium thiosulfate of 0.01 *N* was used in titration, and 1.0 ml. of this reagent was equal to 0.82 mg. of pentosan, the value used by Loska and Shellenberger (13).

This procedure averaged 84.7 ± 2% recovery of D-xylose for 30 determinations over a period of 3 years. Recovery of L-arabinose averaged 66.5 ± 2% for 6 determinations.[3] One ml. of 0.01 *N* sodium thiosulfate was considered equal to 0.75 mg. of pure pentose for calculating recovery of pure pentoses.

Hexoses or hexosans were estimated by determining the total reducing sugars (1) and subtracting the amount of pentoses present. Samples of 0.10 g. were hydrolyzed by refluxing with 50 ml. of 0.5 *N* hydrochloric acid for 2.5 hours (10). Reducing sugars were determined on the filtered, neutralized solution. The sodium thiosulfate was 0.01*N*, and 1.0 ml. of this thiosulfate was equal to 0.34 mg. of glucose, xylose, or arabinose. A factor of 0.9 was used to convert hexoses to hexosans. An analysis of a sample of Elgin prime starch made by this

[3] The yield of furfural from pentoses by reaction with 12% hydrochloric acid and distillation is not quantitative. Wolf *et al.* (24) reported 87% yields from xylose and 81% yields from arabinose. In this work yields of 85% from xylose and 67% from arabinose were obtained. A factor is used to correct for this low yield if estimation of pentosans is made by furfural distillation. Wolf *et al.* used 1.58 times the furfural weight to obtain the anhydro pentose unit. This amounts to a corrective factor of 1.15 for low yield after deduction of the correction from furfural to anhydro pentose unit. A factor of 1.15 would correct an 87% yield to 100%. However, wheat flour polysaccharides contain both xylose and arabinose (19). In this work a corrective factor of 1.24 was used because of the low yields from arabinose. Loska and Shellenberger (13) also used this factor. (1.0 ml. of 0.10 *N* sodium thiosulfate should equal 7.5 mg. of pentose or 6.6 mg. of anhydro pentose unit or 4.8 mg. of furfural; all these quantities are 0.1 meq. Actually 1.0 ml. was taken as equal to 8.2 mg. of anhydro pentose unit or 8.2/6.6 gives a factor of 1.24.)

method gave 101.7% reducing sugars or 91.5% hexosan; the sample had 7.6% moisture and 0.3% protein.

Results

Cookie Diameter Effect of Major Fractions. Previously it had been shown by fractionating and reconstituting techniques that about half of the difference in cookie diameter between a good soft-wheat flour and a poor soft-wheat flour was due to the tailings fraction, and about a fourth was due to the water-solubles. In the present work, a different method of testing for cookie diameter effect was used, and therefore the effect of the four major flour fractions on cookie diameter by this method was determined. The following diameter reductions were obtained when 1.0 g. of the major fraction was added to 40.0 g. of the standard cookie flour:

	From Rio	*From Elgin*
	mm	*mm*
Water solubles	3.1	2.3
Tailings	2.4	3.3
Prime starch	0.5	1.2
Gluten	+0.1	1.2

Effect of Alcohol-Soluble Material. The alcohol-soluble portion of the water-solubles was obtained from several flours, and Table I gives the yields, analyses, and baking results of these preparations. The alco-

TABLE I

EFFECT ON COOKIE DIAMETER OF FRACTIONS FROM WATER-SOLUBLES

Solubility of Fractions in 71% Ethanol and Variety	Yield[a]	Protein	Pentoses or Pentosans[b]	Hexoses or Hexosans[b]	Water Added[c]	Reduction in Cookie Diameter[d]
	g	%	%	%	*ml*	*mm*
Soluble fractions						
Elgin	2.28	17.2	1.5	51.3	0.0	0.2
Elmar	2.46	18.8	1.5	47.9	.0	+ 0.6
Marfed	2.60	18.9	2.0		.0	0.4
Brevor	3.00	16.7	1.8	54.0	.0	0.2
Rio 53	3.08	12.6	1.5	62.4	0.0	+ 0.6
Insoluble fractions						
Elgin	1.01	27.3	36.5	...	1.3	9.2
Elmar	0.97	24.5	36.8	27.6	1.2	9.7
Marfed	0.95	29.2	40.7	18.3	1.2	9.1
Brevor	0.83	32.5	37.6	17.5	1.3	10.9
Rio 53	1.40	20.1	48.7	19.9	1.2	9.7

[a] Grams of the lyophilized fraction "as-is" from 100.0 g. of flour at 14% moisture.

[b] These constituents were assumed to be free sugars in the alcohol-soluble materials and polymers in the alcohol-insoluble materials.

[c] The amount of additional water required to give a dough similar in consistency to the dough from the standard cookie flour.

[d] The decrease in diameter caused by adding 1.0 g. of the substance to the standard cookie flour. A plus sign indicates that the cookie was larger in diameter than the cookie from the standard flour.

hol-soluble fractions, containing 68–78% of the original material, most of the hexoses and protein, and practically no pentoses, had very little effect on cookie diameter. These fractions, when lyophilized, were coarse, buff crystals easily crushed to fine powders. The alcohol-soluble material is assumed to be low-molecular substances such as simple sugars (15), amino acids, and peptides.

The effect of simple sugars on cookie diameter was determined by adding 1.0 g. of a sugar to 40.0 g. of the standard cookie flour and baking cookies from the blend. The effects produced were a reduction of 0.4 mm. for D-xylose and increases of 0.7 mm. for glucose and 0.4 mm. for sucrose. The two monosaccharides caused a dark color and numerous little dark spots in the cookies, but sucrose gave a normal cookie.

Effect of Alcohol-Insoluble Material. The alcohol-insoluble portion of the water-solubles was obtained in the same separations used for the alcohol-soluble fractions. The lyophilized material was a white, fibrous blanket difficult to grind or divide. The results for these fractions are also shown in Table I. The alcohol-insoluble fractions, although they contained only 21–31% of the original water-soluble material, had large diameter effects of 9–11 mm. and had practically all of the original pentose material. These fractions also had a large effect on cookie top grain as shown in Fig. 1. Cookies baked from recon-

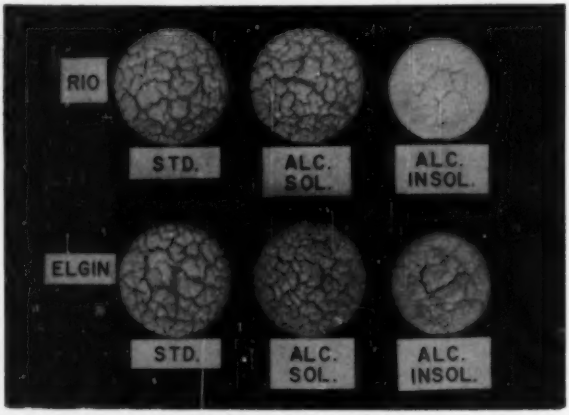

Fig. 1. Effect on cookie diameter of fractions from the water-solubles. STD., cookies from the standard cookie flour (neither Rio nor Elgin). Diameters were 8.98 and 9.04 cm. (different bakes). ALC. SOL., cookies obtained when 1.0 g. of alcohol-solubles was added to 40.0 g. of the standard flour. Diameters were 9.02 cm. for the Rio fraction and 9.01 for the Elgin fraction. ALC. INSOL., cookies obtained when 1.0 g. of the alcohol-insolubles was added to 40.0 g. of the standard flour. Diameters were 8.03 cm. for the Rio fraction and 8.11 for the Elgin fraction.

stituted flours had shown a large effect of the total water-solubles on top grain (21).

The effect of variety is shown in Table I. There appeared to be a normal range of 9–10 mm. for most varieties. Brevor had a somewhat higher effect (10.9 mm.), which was compensated for by a lower yield.

The alcohol-insoluble materials are usually high-molecular, polymeric substances (15,19). The alcohol-insoluble portion of the water-solubles from wheat flour has been termed pentosans by Perlin (19) and Simpson (20). The yields and analytical data of the preparations obtained here were similar to those reported by these workers except that Simpson obtained lower protein contents by heating the water-extract. However, proteins and hexosans were present in amounts nearly as large as the amounts of pentosans, and it was not possible to determine which one, if any, of these three constituents was responsible for the diameter effect. It appeared that a polymeric substance was responsible.

Effect of Refluxing Flour with Ethanol. Several workers observed harmful effects of enzymes on soluble pentosans and proteins (15,17,20). Montgomery and Smith (15) described a method for inactivating enzymes by refluxing with ethanol. This was done before any wetting of flour with water and appears to prevent any possible enzymatic damage.

One part of flour was refluxed with 2.5 parts of 80% ethanol with constant stirring for 30 minutes. After standing for 5 minutes, the supernatant was decanted, and the flour was air-dried. A water-extract

TABLE II

EFFECT OF REFLUXING FLOUR WITH 80% ETHANOL

VARIETY, TREATMENT, AND FRACTION	YIELD[a]	PROTEIN	PENTOSES OR PENTOSANS[a]	WATER ADDED[a]	REDUCTION IN COOKIE DIAMETER[a]
	g	%	%	*ml*	*mm*
ELGIN					
Flour not refluxed					
Alcohol-soluble	2.28	17.2	1.5	0.0	0.2
Alcohol-insoluble	1.01	27.3	36.5	1.3	9.2
Flour refluxed					
Alcohol-soluble	1.75	20.4	1.9	0.0	+0.6
Alcohol-insoluble	0.55	5.9	47.8	1.1	8.1
MARFED					
Flour not refluxed					
Alcohol-soluble	2.60	18.9	2.0	0.0	0.4
Alcohol-insoluble	0.95	29.2	40.7	1.2	9.1
Flour refluxed					
Alcohol-soluble	2.25	29.7	1.6	0.0	+0.9
Alcohol-insoluble	0.50	7.5	59.4	1.2	9.0

[a] See footnotes of Table I.

was prepared from this flour and treated with ethanol to obtain the same fractions as before.

Yields of both fractions (Table II) were lower than those obtained from untreated flour. The alcohol-insoluble fraction had a much lower protein content, a higher pentosan content, and a somewhat lower effect on cookie diameter than similar fractions from untreated flour.

Effect of Heating the Water-Extract. Water-extracts were heated with constant stirring to 94°C. for 4 minutes to inactivate the enzymes (17,20), cooled, and centrifuged to remove the coagulated material. The extract was then poured into ethanol to obtain the same fractions as before. Yields after this treatment were almost as large as those from unheated extracts (Table III). The alcohol-insoluble fractions had

TABLE III
EFFECT OF HEATING THE WATER-EXTRACT

VARIETY AND FRACTION	YIELD[a]	PROTEIN	PENTOSES OR PENTOSANS[a]	WATER ADDED[a]	REDUCTION IN COOKIE DIAMETER[a]
	g	%	%	*ml*	*mm*
RIO 53					
Extract not heated					
Alcohol-soluble	3.08	12.6	1.5	0.0	+ 0.6
Alcohol-insoluble	1.40	20.1	48.7	1.2	9.7
Extract heated					
Alcohol-soluble	2.98	14.0	1.9	0.0	0.5
Alcohol-insoluble	1.24	9.6	52.6	1.1	8.4
Heat-coagulated	0.20	67.2	4.0	0.0	2.8
ELGIN					
Extract not heated					
Alcohol-insoluble	1.01	27.3	36.5	1.3	9.2
Extract heated					
Alcohol-insoluble	0.70	15.5	43.9	1.5	11.6
Heat-coagulated	0.25	73.4	3.3	0.0	4.5

[a] See footnotes of Table I.

lower protein contents, higher pentosan contents, and about the same high effects on cookie diameter as similar fractions from unheated water-extracts.

Effect of Preparations from Barium Hydroxide-Zinc Sulfate Precipitation. Because the effect on cookie diameter appeared to be related, at least partly, to the pentosan content of the fractions, pentosans were prepared by the barium hydroxide-zinc sulfate precipitation method (17). Table IV shows that good yields of nondialyzable materials were obtained from the precipitates, but these materials were lower in protein and pentosans and had less effect on cookie diameter than those precipitated by ethanol.

Effect of Filtrol Treatment. The middle part of Table V presents

yields, analytical data, and baking results of preparations from water-extracts treated with Filtrol according to Pence, Elder, and Mecham (17). Filtrol is an activated clay used to remove proteins from solutions; recovery of the proteins is not possible. Water-extracts treated with Filtrol as in the original method (17) gave good yields of non-dialyzable materials low in protein (Table V). However, inconsistent results were obtained for pentosan contents and effects on cookie diameter. Also, the effect of Filtrol treatment was difficult to separate from the effect of heating the water-extract.

TABLE IV

EFFECT OF PREPARATIONS PRECIPITATED BY BARIUM HYDROXIDE-ZINC SULFATE

VARIETY, TREATMENT OF WATER-EXTRACT, AND METHOD OF PREPARATION	YIELD[a]	PROTEIN	PENTOSANS[a]	REDUCTION IN COOKIE DIAMETER[a]
	g	%	%	mm
RIO 53				
Not heated				
Alcohol-insoluble (for comparison)	1.40	20.1	48.7	9.7
Barium hydroxide-zinc sulfate	1.25	5.9	28.7	2.7
Heated to 94°C.				
Alcohol-insoluble	1.24	9.6	52.6	8.4
Barium hydroxide-zinc sulfate	1.28	1.7	30.3	5.1
ELGIN				
Not heated				
Alcohol-insoluble	1.01	27.3	36.5	9.2
Barium hydroxide-zinc sulfate	1.10	2.6	21.3	3.3

[a] See footnotes of Table I.

TABLE V

EFFECT ON COOKIE DIAMETER OF PREPARATIONS TREATED WITH FILTROL

MATERIAL, VARIETY, AND TREATMENT OF WATER-EXTRACT	YIELD[a]	PROTEIN	PENTOSANS[a]	WATER ADDED[a]	REDUCTION IN COOKIE DIAMETER[a]
	g	%	%	ml	mm
Previous alcohol-insolubles (for comparison)					
Rio 53, not heated	1.40	20.1	48.7	1.2	9.7
Rio 53, heated to 94°C.	1.24	9.6	52.6	1.1	8.4
Elgin, not heated	1.01	27.3	36.5	1.3	9.2
Elgin, heated to 94°C.	0.70	15.5	43.9	1.5	11.6
Marfed, not heated	0.95	29.2	40.7	1.2	9.1
Brevor, not heated	0.83	32.5	37.6	1.3	10.9
Filtrol-treated water extract					
Rio 53, heated to 94°C.	1.18	2.6	43.1	1.2	9.0
Elgin, not heated	1.60	7.0	19.8	0.9	2.0
Elgin, heated to 94°C.	1.28	3.7	22.0	0.7	4.0
Filtrol-treated alcohol-insoluble					
Rio 53, not heated	0.70	8.9	56.3	1.2	11.9
Marfed, not heated	0.44	4.7	64.6	1.2	13.9
Brevor, not heated	0.38	4.1	58.0	1.1	17.3

[a] See footnotes of Table I.

A different method of utilizing Filtrol treatment was to prepare the alcohol-insoluble portion of the water-solubles as was done for Table I. The alcohol-insoluble fraction was then redissolved in water, treated with Filtrol, centrifuged, and dialyzed against distilled water. The bottom part of Table V shows that yields were lower than those of the untreated alcohol-insolubles, but the treated preparations had protein contents of 4–9%, pentosan contents of 56–65%, and diameter effects of 12–17 mm. The treated materials were pure white and fibrous. Comparison of the Filtrol-treated alcohol-insolubles with the untreated alcohol-insolubles showed that both higher pentosan contents and lower protein contents were associated with greater deleterious effects on cookie diameter.

Effect of High-Protein Fractions from Ammonium Sulfate Fractionation. The evidence presented so far suggests a large effect of high pentosan preparations on cookie diameter. The effect of the protein constituents was still obscure. The procedure of Pence and Elder (16) for preparing water-soluble proteins was simplified and used to fractionate water-extracts. The extracts from 700 g. of flour were concentrated to 400 ml. The concentrate was adjusted to 0.4 *M* ammonium sulfate, pH was adjusted to 6.0, and the suspension was stirred for 30 minutes. The precipitate was removed by centrifuging. The supernatant was adjusted to 1.7 *M* ammonium sulfate and treated as before to remove the precipitate. The supernatant at 1.7 *M* and the two precipitates, redissolved in water, were dialyzed against distilled water, shelled, and lyophilized.

Table VI presents the results of the ammonium sulfate fractionations. The 0.4 *M* insoluble materials, corresponding to the gliadin fractions of Pence and Elder, had 86–89% protein, 2–3% pentosans, di-

TABLE VI

EFFECT OF HIGH-PROTEIN FRACTIONS FROM AMMONIUM SULFATE FRACTIONATION

VARIETY AND FRACTION	YIELD[a]	PROTEIN	PENTOSANS[a]	HEXOSANS[a]	WATER ADDED[a]	REDUCTION IN COOKIE DIAMETER[a]
	g	%	%	%	*ml*	*mm*
Elmar						
Precipitated by 0.4 *M*	0.16	89.1	1.7	...	1.1	2.2
Precipitated by 1.7 *M*	0.27	70.2	11.7	...	1.1	4.9
Soluble in 1.7 *M*	0.94	26.7	32.8	26.0	1.0	5.8
Total	1.37					
Golden						
Precipitated by 0.4 *M*	0.27	85.6	2.8	...	1.0	2.7
Precipitated by 1.7 *M*	0.38	57.0	12.5	...	1.1	5.9
Soluble in 1.7 *M*	1.05	26.4	25.5	31.3	0.9	2.3
Total	1.70					

[a] See footnotes of Table I.

ameter effects of 2–3 mm., and required 1.0–1.1 ml. of water. The relatively high amounts of water were not accompanied by large effects on diameter. The 1.7 *M* insoluble materials, corresponding to the albumin fractions of Pence and Elder, had 57–70% protein, 12–13% pentosans, diameter effects of 5–6 mm., and required 1.1 ml. of water. The 1.7 *M* soluble materials had protein, pentosan, and hexosan contents all within the 26–32% range and diameter effects of 2–6 mm.

Effect of Fractional Precipitation by Ethanol. Recovery of all constituents of the alcohol-insoluble fraction was possible in another fractionation procedure. This was step-by-step addition of a second, more selective solvent to a solution of the fraction as employed by Perlin (19), Gilles and Smith (8), and Whistler and Kirby (22). The alcohol-insolubles were prepared from three flours, Rio, Elmar, and Marfed, and redissolved in water to give about 1% solutions. Ethanol was added with stirring to the solutions to give concentrations of 10–70%

TABLE VII

FRACTIONS FROM ETHANOL FRACTIONAL PRECIPITATION

VARIETY AND FRACTION	YIELD[a]	PROTEIN	PENTOSANS[a]	HEXOSANS[a]
	g	%	%	%
Marfed				
Insoluble in 10%	0.10	69.2	8.5	13.9
Insoluble in 20%	.10	71.5	7.5	11.6
Insoluble in 30%	.00	...	...	...
Insoluble in 40%	.01	39.6	...	...
Insoluble in 50%	.17	9.8	68.3	10.5
Insoluble in 60%	.30	7.5	67.7	18.2
Insoluble in 70%	.08	32.3	27.9	28.1
Soluble in 70%	0.23	21.7	18.9	44.2
Total	0.99			
Elmar				
Insoluble in 10%	0.08	71.0	6.1	...
Insoluble in 20%	.02	59.4	9.4	...
Insoluble in 30%	.02	68.4	8.2	...
Insoluble in 40%	.01	48.5	...	...
Insoluble in 50%	.03	29.0	36.6	...
Insoluble in 60%	.40	10.1	62.7	...
Insoluble in 70%	.08	26.4	38.7	...
Soluble in 70%	0.23	19.9	19.3	...
Total	0.87			
Rio 53				
Insoluble in 10%	0.04	37.1	18.9	...
Insoluble in 20%	.11	46.9	17.9	...
Insoluble in 30%	.06	48.4	13.0	...
Insoluble in 40%	.04	35.6	34.8	...
Insoluble in 50%	.58	7.4	65.9	...
Insoluble in 60%	.28	5.2	66.5	...
Insoluble in 70%	.14	26.3	31.1	...
Soluble in 70%	0.25	18.0	15.5	...
Total	1.50			

[a] See footnotes of Table I.

ethanol by 10% steps. The precipitates were removed by centrifuging and air-dried. The material soluble in 70% ethanol was recovered by the same procedure used for the alcohol-solubles.

The results of ethanol fractional precipitation are shown in Table VII. Ethanol concentrations of 10–40% gave moderate yields of fractions high in protein and low in pentosans. Ethanol concentrations of 50 and 60% gave high yields of fractions high in pentosans and low in proteins. Ethanol concentrations of 70% gave moderate yields of fractions with both protein and pentosan contents in the 20–40% range. A considerable portion of the original alcohol-insoluble material was soluble in 70% ethanol following reprecipitation; this material had both protein and pentosan contents in the 18–22% range.

Effect of Fractions from Ethanol Fractional Precipitation. The fractionation procedure was modified to yield enough material for baking tests by grouping the precipitates at 10–40% ethanol, since these were all high-protein, low-pentosan fractions. Likewise, the fractions insoluble and soluble at 70% ethanol were combined because they represented intermediate ranges of protein and pentosan contents.

The alcohol-insolubles from the water-solubles of three flours were prepared and redissolved in water. Ethanol was added in steps to give concentrations of 40, 50, and 60% ethanol. After each addition, the

TABLE VIII

EFFECT OF FRACTIONS FROM ETHANOL FRACTIONAL PRECIPITATION

VARIETY AND FRACTION	YIELD[a]	PROTEIN	PENTOSANS[a]	HEXOSANS[a]	WATER ADDED[a]	REDUCTION IN COOKIE DIAMETER[a]
	g	%	%	%	*ml*	*mm*
Marfed						
Insoluble in 40%	0.22	67.6	18.3	...	1.0	4.2
Insoluble in 50%	.26	5.5	74.3	14.9	1.2	14.7
Insoluble in 60%	.13	14.4	64.8	16.7	1.1	18.3[b]
Soluble in 60%	.29	27.3	18.9	45.5	0.6	+ 0.6
Total	0.90					
Elmar						
Insoluble in 40%	0.15	55.6	17.0	...	1.1	8.2[b]
Insoluble in 50%	.07	34.4	40.2	...	1.2	16.2[c]
Insoluble in 60%	.36	9.3	64.0	...	1.0	12.0
Soluble in 60%	.34	23.9	19.5	...	0.6	+ 0.6
Total	0.92					
Rio 57						
Insoluble in 40%	0.29	37.2	30.6	...	1.0	10.0
Insoluble in 50%	.60	10.3	59.5	...	1.0	12.3
Insoluble in 60%	.15	6.0	56.2	...	1.1	15.1[b]
Soluble in 60%	0.37	22.9	18.5	...	0.5	+ 0.4
Total	1.41					

[a] See footnotes of Table I.
[b] Only enough material available for baking two samples.
[c] Only enough material available for baking one sample.

precipitates were removed, dissolved in water, shelled, and lyophilized. The supernatant at 60% ethanol was concentrated as previously described, shelled, and lyophilized.

The results (Table VIII) show a large varietal influence on this fractionation procedure. Marfed gave the best separations, and Rio gave the poorest.

One common result from all three fractionations was the ineffectiveness of the 60% soluble materials, although they contained moderate amounts of proteins and pentosans. The high-protein fractions (40% insoluble materials) had diameter effects of 4–10 mm., and the high-pentosan materials (50 and 60% insoluble materials) had effects of 12–16 mm.

The high-pentosan fractions were similar in pentosan content and cookie diameter effect to the preparations obtained by Filtrol-treatment of alcohol-insolubles (Table V). As little as 0.5 g. of these high-

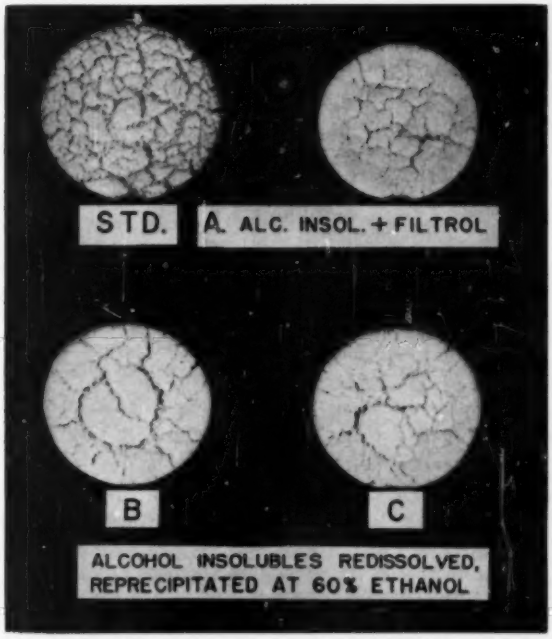

Fig. 2. Effect on cookie diameter of preparations high in pentosans. STD., cookie from a standard cookie flour. A, cookie from this flour plus 0.5 g. of Marfed alcohol-insolubles treated with Filtrol (Table V). B and C, cookies from this flour plus 0.5 g. of the material precipitated at 60% ethanol concentrations from redissolved alcohol-insolubles; B, from Rio; C, from Marfed (Table VIII).

pentosan materials would reduce cookie diameter greatly as shown in Fig. 2.

Discussion

The low-molecular constituents of the water-soluble fraction had only very slight effects on cookie diameter. These slight effects were shown by both the original alcohol-solubles and the materials soluble in 60% ethanol upon reprecipitation. The latter materials were presumably low-molecular constituents occluded in the first alcohol precipitation. These slight effects were often increases in diameter and were an example of a quality factor that did increase diameter. Sucrose is known to increase cookie diameter, and the increases caused by some alcohol-soluble fractions may be due to the sugars present because hexoses predominate in this fraction. The dark color caused by the monosaccharides was presumably due to the browning effect. The large effects on diameter, however, were due to the higher-molecular or polymeric constituents and were decreases in diameter.

The water-soluble proteins had small to moderate effects on diameter. Ammonium sulfate fractionation probably gave the best preparations for evaluating the effects of proteins as these preparations were very low in pentosans and were free from any possible alcohol denaturation. The high-protein fractions from fractional ethanol precipitation had pentosan contents over 17%; part of the effects of these preparations on diameter may have been due to the pentosans present, and the pentosans also made it difficult to determine if any alcohol denaturation occurred. More clearly separated fractions might have been obtained by fractional precipitation if the ethanol had been added in 10% (or less) steps as in the original procedure and a larger batch of flour had been employed as starting material to obtain sufficient fractions for baking tests. However, a rather minor part of the effect of the water-solubles on diameter was probably due to the proteins because of the moderate effects coupled with the relatively small amounts of proteins in the polymeric material.

The water-soluble polysaccharides had a large effect on both cookie diameter and top grain. These fractions were high in pentosan content. Hexosans were present, but it was not possible to determine their effect. Most of the effect of the water-solubles on cookie diameter was due to the polysaccharides, particularly the pentosans.

The relation between total flour pentosans and cookie diameter was determined for 45 samples from 3 crop years. A correlation coefficient of -0.49 (5% pt. $= 0.30$) was found. For the same samples, a correlation coefficient of -0.79 was found between alkaline water reten-

tion capacity values and cookie diameter. Two possible explanations for the low correlation with flour pentosans are that the soluble pentosans comprised only about 20% of the total pentosans, and the effect of the other 80% was unknown, and that the effects of the tailings fraction overshadowed the effects of the water-solubles.

In this study no evidence of any enzyme activity harmful to cookie quality factors was found. The polysaccharides appeared unharmed when heated in water solution to 94°C.

The preparations obtained by the barium hydroxide-zinc sulfate precipitation had low pentosan contents and small diameter effects. Pence, Elder, and Mecham (17) concluded from molecular weight and ultracentrifuge determinations that this reagent precipitated the larger pentosan particles as well as the proteins of the water-extract. The lower pentosan contents and smaller diameter effects of the preparations obtained here supported this conclusion.

The water required to form a normal dough when 1.0 g. of the water-soluble constituent was added to the standard flour is listed in most tables of this study and for most samples was closely related to the effects on diameter. This close relation supported the conclusion of Yamazaki (25) that flour components absorbing large amounts of water will have a detrimental effect on cookie diameter. Two exceptions to this close relation were the large amount of water required by soluble high-protein fractions with small diameter effects (Tables VI, VIII) and the failure of high-pentosan preparations with high effects on diameter to require more than the usual 1.1–1.2 ml. of water (Tables VI, VIII).

Flour pentosans have been shown to have no effect on bread loaf volume (17) and to cause difficulties in starch manufacture (4). There appears to be an inverse relation between acid-extracted pentosans and milling score (5). In this work, pentosans were shown to have a detrimental effect on baking quality of cookies. Apparently, pentosans are undesirable in wheat and flour, and wheat varieties lower in pentosan content are desirable.

Further clarification of the role of pentosans in dough handling (2,17) and flour mixing (14) is necessary before their undesirability can be assured. It also appears that the quality factors in cookie-baking act in a sort of regulatory manner. Yamazaki (25) showed that a flour reconstituted with no tailings yielded an abnormally large cookie of very poor appearance. Good-quality cookie flours have small to moderate amounts of tailings and water-solubles; these bring the diameter down to about 9.0 cm. and cause an attractive appearance. Poor-quality cookie flours have larger amounts of tailings and water-solubles

which further decrease the diameter to about 8.0–8.5 cm. and cause a poorer appearance. Complete elimination of deleterious factors thus may not be desirable.

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SOME ASPECTS OF THE SULFHYDRYL-DISULFIDE SYSTEM IN FLOUR AND DOUGH[1]

H. MATSUMOTO AND I. HLYNKA

ABSTRACT

The sulfhydryl content of flour and dough extracts was determined amperometrically with silver nitrate, using a tris buffer medium and a convection platinum electrode in a newly designed airtight titration vessel. The mixing of dough and the preparation and titration of extracts were done in an atmosphere of nitrogen. The results showed that on the basis of 100 g. protein the water-soluble components of flour contained two to three times as much sulfhydryl as the fraction soluble in 0.01*N* acetic acid (largely gluten proteins). The ratio of free sulfhydryl to disulfide, however, tended to remain constant. The sulfur-containing lipoproteins extracted with petroleum ether contained negligibly small amounts of free sulfhydryl. In dough mixed in air, or treated with bromate or with iodate, the water-soluble fraction showed a definite but not a marked decrease in the sulfhydryl content. The acid-soluble fraction from the same doughs showed that atmospheric oxygen had no significant effect, but other oxidants decreased the sulfhydryl content. Sulfhydryl-blocking agents, *N*-ethylmaleimide and *p*-chloromercuribenzoate, added to dough had a marked effect in reducing the sulfhydryl content of both the water-soluble and acid-soluble fractions.

The probable role of the sulfhydryl radical in dough chemistry has attracted the attention of many cereal chemists. The main premise has been that the sulfhydryl radical is potentially capable of undergoing a cross-linking reaction to form disulfide bridges between protein chains. Such a reaction would then be expected to have an important effect on the rheological properties of dough. In fact, it has been suggested

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as a chemical basis of the action of improving agents such as bromate and iodate (10,24).

Progress in the study of the chemistry of the sulfhydryl radical in flour and dough has, however, been hampered by limitations of the analytical methods available. Only a minute amount of free sulfhydryl is present in flour and methods for its estimation must be extremely sensitive and precise. Moreover, extracts of flour and dough contain small amounts of readily oxidizable substances which interfere in those analytical methods that depend upon an oxidizing agent. Nevertheless, Meyers and Working (19) and Baker, Parker, and Mize (1) used ferricyanide, and Holme and Spencer (11) and Nordin and Spencer (20) used *o*-iodosobenzoic acid as the oxidant with considerable success. These investigators showed that starch may also be an interfering substance.

The method introduced by Benesch and Benesch (3) and by Kolthoff, Stricks, and Morren (14), which depends upon the combination of silver or mercury salts with the sulfhydryl group, obviates the difficulties inherent in the use of oxidizing agents. The precision of the titration was also increased by the use of the amperometric technique. Matsumoto (16) and Matsumoto and Shimoda (18) used urea to solubilize wheat proteins for the sulfhydryl titration, as did Benesch, Lardy, and Benesch (4) who further added tris buffer to carry out the titration at pH values nearer to those encountered in materials of biological origin.

With the amperometric method Matsumoto (16) and Matsumoto and Shimoda (18) determined the sulfhydryl content of washed-out, dispersed gluten; Sullivan (24) studied the water-soluble fraction of flour; and Kong, Mecham, and Pence (13) studied the water-soluble fraction of flour and of tryptic digests of gluten.

The present paper reports an adaptation after Koide and Kida (12) of the convection platinum electrode, and describes a procedure that makes it possible to mix doughs, to make extractions and dispersions, and to titrate water-soluble and acid-soluble (gluten) proteins in an atmosphere of nitrogen. This method was applied to an examination of several aspects of the chemistry of the sulfhydryl radical in dough.

Materials and Methods

Flour. The flour used in this study was an unbleached, improver-free, straight-grade flour commercially milled from a blend of Canadian hard red spring wheat. It had 13.1% protein and 0.50% ash, on a 14% moisture basis. Its response to bromate and iodate, and its behavior in baking were in no way anomalous.

Mixing of Dough. Doughs were mixed in a GRL mixer with a gas-tight bowl in a nitrogen atmosphere (9) for 2.5 minutes except for oxygen treatment in which air was substituted for nitrogen. The absorption used was 60%. Bromate, iodate, and other reagents in solution were added to flour during the preparation of the dough. For the experiments with bromate-treated doughs a reaction time of 3 hours was given for the dough before extraction and analysis. For doughs treated with other reagents, extraction and analysis were carried out directly.

Fractionation into Water- and Acid-Soluble Components. In order to study the sulfhydryl-disulfide distribution in the water-soluble and the acid-soluble or gluten components, the flour and the dough were fractionated. The procedure was essentially that of Lusena (15), Cunningham, Geddes, and Anderson (6), and Sollars (23). All extractions were carried out at a room temperature of about 22°C.

To obtain the water extract, 40 g. flour that had been equilibrated in a nitrogen atmosphere overnight (or dough equivalent to 40 g. flour mixed in nitrogen taking water into account) were dispersed with 320 ml. oxygen-free water in a Waring Blendor also in nitrogen under an air-tight bell jar. After 2 minutes of mixing, the dispersion was left for 30 minutes to allow for equilibrium between phases. The slurry was further mixed for 1 minute and then centrifuged for 15 minutes at 1,800 r.p.m. in an International centrifuge. The supernatant obtained in this way was the first water extract and had a pH of 6.0 and represented a water-to-flour ratio of 8:1.

A second water extract was obtained from the residue of the first extraction with 280 ml. oxygen-free water. No rest period was used in this extraction. The extract had a pH of 5.0 and represented a water-to-flour ratio of 7:1. This second extract was intended primarily to ensure completeness of the first extraction and to make the distinction between the first and third extracts more definite. Further fractionation (22) with 0.4*M* ammonium sulfate and heat coagulation gave for the first extract values of 29% albumin, 31% globulin, and 40% gliadin, and for the second extract 73% gliadin. This confirmed that the second extract was taking out mainly the gliadin portion of the gluten proteins.

A third extract was obtained by using 200 ml. of 0.01*N* acetic acid on the residue of the previous extraction. A period of 30 minutes in the Waring Blendor was allowed to complete changes due to swelling in the acid solution. The pH of the extract was 4.4 and represented a water-to-flour ratio of 5:1.

A fourth extract was also made with 120 ml. of 0.01*N* acetic acid,

had a pH of 4.0, and represented a water-to-flour ratio of 3:1. The fourth extract served to indicate the completeness of the previous extraction. The residue from the fourth extract was assayed in urea dispersion.

As a further check on the water extractions, which were too dilute to yield precise titrations, a more concentrated water extract was also obtained by using a flour-to-water ratio of 1:2. This procedure provided data supplementary to those obtained with dilute extracts. The pH was 5.9. For studies on dough only one water extract and one acid extract were used.

Amperometric Titration. The titration vessel, a modification of Koide's convection platinum electrode (12), permitted evacuation of the system and flushing it with nitrogen during the titration. The main features are illustrated in Fig. 1. The composition of the electrolyte was that used by Benesch, Lardy, and Benesch (4) except that the final concentration of urea was 4.8 molar. The titration was carried out in an atmosphere of nitrogen with 0.05*N* silver nitrate, with constant stirring of the electrolyte by a magnetic stirrer. The electrochemograph (Leeds and Northrop) was used as a sensitive microam-

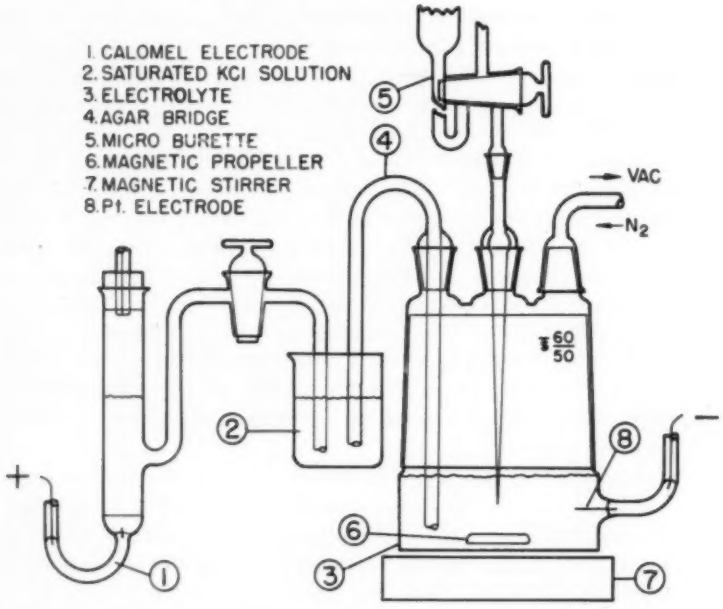

Fig. 1. Assembly for amperometric titration in an atmosphere of nitrogen.

meter. The reliability of the method was checked against a sample of glutathione of known titer. The results are reported in moles of –SH per 100 g. flour and also as moles per 100 g. protein based on the nitrogen content of individual fractions, and represent the mean of at least two separate experiments.

Cystine Determination. Samples containing about 400 mg. protein in 6*N* hydrochloric acid were purged with nitrogen, evacuated, and sealed in glass tubes. Digestion was carried out at 108°C. for 20 hours with occasional inversion of the tubes. The digestion product was filtered and cystine content was determined by a modified Okuda's method (7,21) which is based on reduction with zinc and titration with iodine solution. The method was tested by recovery of cystine added to the wheat proteins. The results are reported in moles of S-S per 100 g. protein.

Extraction with Petroleum Ether. A large Soxhlet apparatus was used for extraction with petroleum ether (boiling range 90° to 95°F.). The extraction was continued for 20 hours with a stream of nitrogen through the system.

Results

The results are presented in three sections. Data on the distribution of the sulfhydryl and disulfide group in various flour fractions are summarized first. Then follow observations on the effect on the sulfhydryl group of oxygen, bromate, iodate, and sulfite. These reagents represent common oxidizing improving agents and a reducing agent whose reaction in dough has been fairly extensively studied. Recently there has been an interest in the possibility of obtaining additional information by the use of sulfhydryl-blocking agents: accordingly, some results on the effect of *N*-ethylmaleimide and *p*-chloromercuribenzoate have also been included.

Distribution of the Sulfhydryl Group and Disulfide Linkage in Flour Fractions. Table I summarizes the data obtained for the sulfhydryl and disulfide content of water-soluble components and of components of flour soluble in dilute acetic acid. The data show that the third extract, which contains most of the flour proteins, also contains most of the sulfhydryl as proportion of total. However, on the basis of 100 g. protein the first extract contains more than twice as much sulfhydryl as the third extract. A more precise determination made on a concentrated extract (low water-to-flour ratio) gave an even higher result. Data on cystine content show that the disulfide linkage is highest in the first extract, which also has the highest sulfhydryl content. The ratio of free sulfhydryl to disulfide tends to remain constant.

TABLE I

DISTRIBUTION OF SULFHYDRYL GROUP AND DISULFIDE LINKAGE IN FLOUR FRACTIONS

	Medium	Nitrogen Distribution (Percent of Total)	–SH Group (per 100 g. Flour) $\times 10^{-6}$ mol.	–SH Group (per 100 g. Protein) $\times 10^{-4}$ mol.	S–S Linkage (per 100 g. Protein) $\times 10^{-2}$ mol.
1st Extract	Water	11.4	12.8	8.6	1.19
2nd Extract	Water	9.9	3.8	2.9	0.80
3rd Extract	Acid	58.2	29.0	3.8	0.80
4th Extract	Acid	7.4	4.4	4.5	0.73
Residue	.....	7.2	6.2	6.6	...
Total	.....	94.1	56.2	...	...
Concentrated extract	Water	10.4	...	13.0	...

In addition to the experiments reported in Table I, two supplementary experiments were carried out. In the first, gluten was washed out by hand in water through which nitrogen was bubbled freely. This gluten dispersed in 0.01*N* acetic acid gave 3.9×10^{-4} moles of –SH per 100 g. protein. This value is quite close to that of the third extract in which gluten proteins were extracted directly. In the second experiment, a petroleum ether extract which has been shown (2) to contain sulfur-containing lipoproteins, was prepared. The sulfhydryl content of this extract was 10^{-7} moles –SH per 100 g. flour. This amount is considered to be negligible in comparison with the amounts of –SH found in other fractions shown in Table I.

Effect of Atmospheric Oxygen, Bromate, Iodate, and Sulfite. Results on the effect of atmospheric oxygen, bromate, iodate, and sulfite on the sulfhydryl group of the water-soluble (concentrated water extract) and acid-soluble components of flour and dough are summarized in Table II. The first two entries show that very nearly the same results were obtained whether the determination was carried out on flour or on dough. In the water-soluble fraction, atmospheric oxygen, bromate (3 hours' reaction time), and iodate all decreased the sulfhydryl content. In the acid-soluble or gluten fraction, atmospheric oxygen had no significant effect, but other oxidants decreased the sulfhydryl content as before. Sodium sulfite by itself does not titrate in the amperometric titration. However, it reacts readily with the water-soluble components to give high sulfhydryl values. The results on the acid-soluble or gluten fraction are but little influenced by the sulfite.

Effect of Sulfhydryl-Blocking Agents. Table III summarizes the data on the effect of the sulfhydryl-blocking agents N-ethylmaleimide and *p*-chloromercuribenzoate. The amount of the reagents used was about five times the theoretical amount based on the titration value

TABLE II

EFFECT OF SOME OXIDIZING AND REDUCING AGENTS ON THE SULFHYDRYL GROUP OF WATER- AND ACID-SOLUBLE FRACTIONS

SOURCE OF SAMPLE	-SH GROUP CONTENT ($\times 10^{-4}$ mol. per 100 g. protein)	
	Water-Soluble Fraction (conc. extract)	Acid-Soluble Fraction
Flour	13.8	3.80
Dough		
Mixed in nitrogen atmosphere	13.4	3.59
Mixed in air atmosphere	11.7	3.46
Mixed with 30 p.p.m. of potassium bromate	11.5	2.48
Mixed with 20 p.p.m. of potassium iodate	11.7	2.93
Mixed with 250 p.p.m. of sodium sulfite	16.4	3.79

TABLE III

EFFECT OF SULFHYDRYL-BLOCKING AGENTS ON THE SULFHYDRYL GROUP OF WATER- AND ACID-SOLUBLE FRACTIONS OF DOUGH[a]

SAMPLE	-SH GROUP CONTENT ($\times 10^{-4}$ mol. per 100 g. protein)		
	Control	N-ethyl-maleimide	*p*-chloromercuri-benzoate
Water-soluble fraction (concentrated extract)	14.5	5.5	3.5
Acid-soluble fraction	4.0	2.4	2.1

[a] 3.0×10^{-4} mol. of N-ethylmaleimide and of *p*-chloromercuribenzoate were used for 100 g. flour.

for the control experiment. In these experiments the doughs were mixed in the GRL mixer under nitrogen for 10 minutes.

The results show that N-ethylmaleimide decreased the sulfhydryl content of the water-soluble (concentrated water extract) components by 62% and of the acid-soluble components by 40%. Corresponding decreases with *p*-chloromercuribenzoate were 76 and 48%. About a third of the sulfhydryl, as shown by amperometric titration, remained unreacted. Attempts to react the remaining sulfhydryl by increasing the amount of reagents, the reaction time, and the mixing time were unsuccessful. In experiments with gluten dispersions to which ammoniacal buffer was added directly, Matsumoto (16) obtained apparently complete reaction with *p*-chloromercuribenzoate.

Discussion

In considering the results as a whole, the following features may be noted. In the present work precautions were taken to exclude atmospheric oxygen in the preparation of the samples and during titration. The difference found between the sulfhydryl values for the water-soluble components from doughs mixed in air, and in nitrogen

(Table II), shows a small but definite effect that may be attributed to oxygen.

The data on gluten were obtained mainly on acid extracts of dough and flour, but confirmatory experiments were also made on gluten washed out by hand in water through which nitrogen was bubbled freely. Definite values for the sulfhydryl content of gluten were established. A relatively high sulfhydryl content in the water-soluble components of dough and flour was also confirmed.

The reaction of bromate and iodate resulted in small but definite decreases in the sulfhydryl content of both the water- and acid-soluble components of dough. The effect of oxygen on the acid-soluble or gluten fraction was doubtful. In contrast, the sulfhydryl-blocking agents decreased the sulfhydryl content of dough very markedly. The action of the reducing agent sodium sulfite readily increased the free sulfhydryl content of the water-soluble components; the effect on the acid-soluble components was inappreciable and may indicate a reversibility of the reaction upon removal of the reactant in a preliminary water extraction. These results are in harmony with earlier studies of Hlynka (8) and of Matsumoto (17).

In general, the results of the present study are not inconsistent with the hypothesis that dough improvers may act to oxidize the gluten proteins. On this basis the water-soluble proteins should also have a significant role. However, the possibility that the oxidation of the sulfhydryl group is an adventitious reaction cannot be entirely overlooked. Other important questions, such as the effect on the physical properties of dough of monofunctional reagents represented by N-ethylmaleimide, also remain unexplained. Further aspects of the significance of the sulfhydryl radical in cereal chemistry have been discussed in a very recent paper by Bloksma (5). Recent development of new techniques and new reagents promises more rapid progress in the chemistry of the sulfhydryl radical in dough.

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THE FATE OF VARIOUS SUGARS IN FERMENTING SPONGES AND DOUGHS[1]

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ABSTRACT

In sponges set at 25°, 30°, and 37°C., covering the normal range of temperatures used commercially, the glucose and fructose naturally present in the flour were fermented simultaneously, although glucose was utilized more rapidly. The concentration of maltose in the sponges increased until the levels of glucose and fructose were reduced to a low value; thereafter the maltose concentration decreased rapidly. Two gas production maxima were observed in fermenting sponges; the first occurred as a result of rapid fermentation of the monosaccharides; the second appeared after the yeast had become adapted to rapid maltose fermentation.

Regardless of whether 5% (flour basis) glucose, fructose, sucrose, or maltose was added at the dough stage, the maltose concentration decreased continuously during dough-fermentation. Doughs with no added sugar or with 5% added maltose made from sponges set at 25° or 30°C. produced significantly more gas during the first 40 minutes of fermentation than did doughs containing 5% added glucose, fructose, or sucrose. In the following 60-minute pan-proof period the doughs containing added sugars produced more gas than those made without added sugar.

Sponges set at 25°C. made with 2.25% yeast (total flour basis) and fermented for 300 minutes produced considerably more gas than did sponges containing 2.0% yeast set at 37°C. and fermented for 250 minutes. This indicated that the yeast levels and fermentation times employed were not properly adjusted to give sponges with the same degree of maturity after fermentation at the two temperatures. Doughs made from sponges fermented at 25°C. proofed more rapidly than did those made from sponges set at 37°C., especially when maltose was the sugar added at the dough stage.

Koch, Smith, and Geddes (4) studied the influence of various added sugars on the rate of disappearance of individual sugars in fermenting straight doughs. They found that glucose was utilized slightly faster than fructose, except when glucose was present in very low concentration. Sucrose was inverted completely when the first samples were taken for analyses 7 minutes after mixing. When glucose, fructose, and maltose were present in the fermenting system, the concentration of maltose increased until the supply of glucose and fructose was exhausted. At this point there was a temporary lag in the gas-production rate until the yeast developed the ability to ferment maltose rapidly. In limited studies on the sponge and dough process they found that yeast became adapted to rapid maltose fermentation during the sponge

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stage. In doughs made from these sponges, the maltose concentration decreased continuously even in the presence of relatively high concentrations of glucose and/or fructose.

There have been several studies on the concentrations of individual sugars in bread. Rice (7) was the first investigator to do any extensive work in this field. He found that when sucrose was added to dough, the finished bread contained more fructose than glucose. Rice (7) concluded that in sponges and doughs, glucose was the preferred substrate for yeast and, moreover, that glucose inhibited the production of maltose. Bohn (1) analyzed commercial breads for the concentration of individual sugars. In the 18 samples he examined, the total sugar content ranged from 2.36 to 5.65% on a crumb moisture basis. Bohn suggested that a low level of maltose in the finished loaf was a good indication that the bread had been made by the sponge and dough procedure. Koch, Smith, and Geddes (4) found no glucose or fructose in bread made from a straight dough containing 2.5% added sucrose. When higher percentages of sucrose were employed, both glucose and fructose were found in the finished bread, but the fructose concentration was much higher than that of glucose. Maltose was found in all breads analyzed, although the concentration in the loaves made by the sponge and dough process was much lower than in those made from straight doughs. Griffith and Johnson (3), using paper-chromatographic techniques, found that when the sponge and dough process was used, the maltose level in the bread was as high as or higher than that originally present in the dough if glucose, fructose, or sucrose was added. When maltose was added at the dough stage, the maltose level in the bread was much lower than that present in the freshly mixed dough.

The present paper describes the results of sugar determinations made on fermenting sponges, doughs, and breads with various sugars added at the dough stage. To determine the relative merits of "hot," "normal," and "cool" sponges, sponges were set at 25°, 30°, and 37°C., a range which covers the temperatures used commercially. Also presented are the results obtained from gas production studies, made with the Zymotachygraphe, on the fermenting sponges and doughs.

Materials and Methods

Preparation of Sponges, Doughs, and Bread. For the preparation of sponges and doughs, a Southwestern Baker's Patent flour (protein 11.8%, ash 0.43% at 14% moisture) was used. Quantitative paper-chromatographic analysis showed it to contain glucose (0.02%), fructose (0.05%), sucrose (0.15%), and maltose (0.12%).

The sponge and dough formulas employed in these studies were as follows:

Ingredient	*Quantity*			
	Sponge Temperature			*Dough*
	25°C.	*30°C.*	*37°C.*	
	g	*g*	*g*	*g*
Flour	70.0	70.0	70.0	30.0
Yeast, compressed	2.25	2.0	2.0	...
Yeast food[a]	0.5	0.5	0.5	...
Nondiastatic malt extract	...	...	...	0.5
Sugar	...	...	...	0 or 5.0
Sodium chloride	...	...	...	2.0
Nonfat dry milk	...	...	...	5.0
Shortening (hydrogenated vegetable)	...	...	...	3.0
Water	variable	variable	variable	variable

[a] A commercial yeast food and dough conditioner containing ammonium salts and potassium bromate as the principal active ingredients.

For each sponge or dough, twice the above quantities were employed. The sponge ingredients were mixed for 1 minute in a Swanson mixer fitted with a McDuffee bowl, then allowed to ferment at 25°, 30°, or 37°C. (77°, 86°, or 98.6°F.) for 5, 4, and 4 hours respectively. These temperatures and other experimental conditions cover the range normally encountered in commercial practice with "cool," "normal," and "hot" sponges. The doughs made from the sponges set at 30°C. were mixed for 3 minutes in a Swanson mixer fitted with a McDuffee bowl and fermented for 30 minutes at 30°C. After scaling to 150 g., molding and panning, the doughs were proofed for 60 minutes at 30°C., then baked for 25 minutes at 232°C. (449.6°F.). The doughs made from the sponges set at 25°C. and 37°C. were mixed for 3 minutes and fermented at 37°C. for 100 minutes but were not baked.

Sponges and doughs prepared in this way were used for the determination of sugar levels and gas production.

Chromatographic Determination of Individual Sugars in Sponges, Doughs, and Bread. The procedure for the removal and chromatographic separation of individual sugars in sponges, doughs, and bread was the same as that described by Lee and Geddes (5). The quantities of sugar eluted from the various strips were determined by the phenol-sulfuric acid method of Dubois *et al.* (2); allowance was made for the amount of sugar on the strips used for the location of the sugars.

Gas Production. Gas production in sponges and doughs was meas-

ured using the Chopin Zymotachygraphe. This instrument provides a continuous record of the gas production and retention of a fermenting dough placed in the fermentation chamber of the apparatus. In this study, only the gas production data were used. The sponge was prepared from 105 g. of flour and an additional 45 g. were added, together with the other ingredients, at the dough-mixing stage. The sponges and doughs were fermented in the Zymotachygraphe at 25°, 30°, or 37°C. The gas-production data were recorded in duplicate over a 6-hour period and were corrected to standard temperature and pressure (0°C. and 760 mm. mercury).

Results and Discussion

Changes in the Concentration of Individual Sugars During Fermentation of Sponges and Doughs at 30°C. The mean results of duplicate sugar determinations on sponges and on doughs fermented at 30°C. to which 5% levels of glucose, fructose, sucrose, and maltose were added at the dough stage are given in Table I. Included also are the values obtained for a sponge and dough made without any added sugar, and the results of sugar determinations on the bread made from each sample. Lactose was not fermented in any of the doughs and remained at its initial level in the dough throughout the dough fermentation (approximately 1.55% at 45.2% dough moisture). The lactose levels have not been included in Table I.

The results of these sugar determinations show that glucose was fermented in the sponge somewhat more rapidly than was fructose. The maltose content of the sponge when sampled 4 minutes after mixing was 1.11%, dough basis, or 1.74% on a flour basis (14% moisture). This represents a 14.5-fold increase over the original concentration of maltose in the flour. A similar finding was reported by Koch *et al.* (4) in their studies on straight doughs. Lee and Geddes have carried out experiments to ascertain the nature of the sudden increase in maltose content of a sponge or dough upon mixing, and the results are reported in a companion paper (6). The concentration of maltose in the sponge increased slightly for the first 90 minutes, during which time glucose and fructose were present. After 90 minutes, the supply of glucose and fructose was exhausted and the concentration of maltose then decreased rapidly to the end of the 4-hour fermentation period. In the dough, the glucose, fructose, and maltose concentrations all decreased from the time of mixing. Glucose and fructose had a sparing effect on the fermentation of each other. The observation that the maltose concentration decreased continuously in the dough, regardless of the sugar added, is in accord with the findings of Koch,

Smith, and Geddes (4). They suggested that yeast became adapted to the rapid fermentation of maltose during the sponge stage and it retained this property in the dough even when relatively high concentrations of glucose or fructose were present.

Gas Production Studies at 30°C. Representative gas production data obtained with the Zymotachygraphe on sponges and doughs fermented at 30°C. are given in Fig. 1.

With the sponges, the gas production rate rose for the first 30 minutes, then dropped; at about 70 minutes it increased once more. The

TABLE I

CHANGES IN SUGAR CONCENTRATION DURING FERMENTATION AT 30°C. OF SPONGES, DOUGHS, AND BREADS CONTAINING VARIOUS SUGARS ADDED AT THE DOUGH STAGE[a]

(Yeast 2.0%, total flour basis)

	FERMENTATION TIME	GLUCOSE	FRUCTOSE	MALTOSE
	minutes	%	%	%
		Control, no added sugar		
Sponge	4	0.30	0.46	1.11
	90	0.11	0.32	1.24
	180	0.00	0.00	0.24
	235	0.00	0.00	0.05
Dough	6	0.12	0.14	0.28
	30	0.09	0.10	0.25
	90	0.00	0.00	0.14
Bread		0.00	0.00	0.28
		5% Glucose added		
Dough	6	2.70	0.10	0.38
	30	2.60	0.14	0.36
	90	1.75	0.12	0.20
Bread		1.53	0.10	0.15
		5% Fructose added		
Dough	6	0.19	2.74	0.45
	30	0.17	2.45	0.34
	90	0.11	2.20	0.16
Bread		0.10	1.66	0.10
		5% Sucrose added		
Dough	6	1.55	1.52	0.47
	30	1.46	1.45	0.40
	90	1.18	1.37	0.19
Bread		0.98	1.21	0.18
		5% Maltose added		
Dough	6	0.08	0.12	2.56
	30	0.08	0.13	2.22
	90	0.00	0.00	2.08
Bread		0.00	0.00	1.79

[a] Sugar concentrations are expressed as a percentage of the sponge or dough weight computed to a moisture content of 45.2%.

dip in gas production does not coincide with the point at which the supply of glucose and fructose was exhausted in the identical sponge (Table I). It was not until some time between 90 and 180 minutes of fermentation that the supply of glucose and fructose was completely utilized. It would seem that between 30 and 70 minutes their concentrations became limiting, and that after this time the yeast became adapted to the rapid fermentation of maltose.

The gas production by sponges and doughs containing various sugars added at the dough stage was determined by measuring the area under the duplicate curves with a planimeter. The values so obtained are given in Table II.

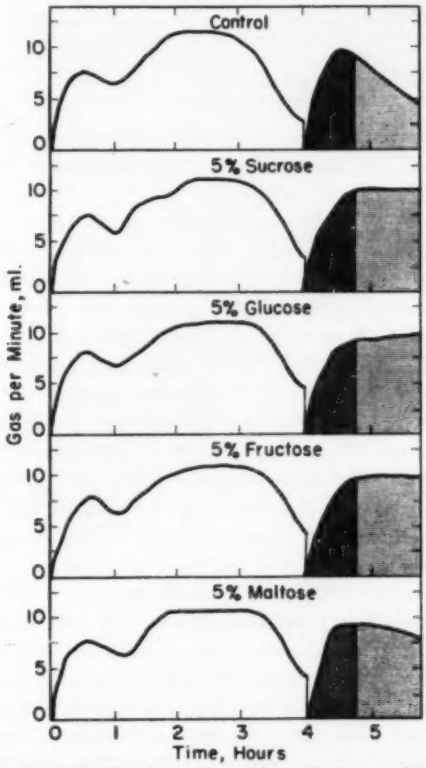

Fig. 1. Rate of gas production in sponges (0 to 4 hours) and in doughs (4 to 5.7 hours) containing 2.0% yeast (total flour basis), fermented at 30°C. The sugars were added at the dough stage. The cross-hatched and horizontally hatched areas represent the gas production in doughs during the first 40 and succeeding 60 minutes respectively.

TABLE II

GAS PRODUCTION IN SPONGES AND DOUGHS AT 30°C. CONTAINING VARIOUS SUGARS ADDED AT THE DOUGH STAGE

(Yeast content was 2.0%, total flour basis)

SUGAR ADDED[a]	GAS PRODUCTION[b]		
	Sponge (4 Hours)	Dough (40 Minutes)	Pan Proof (60 Minutes)
	ml	*ml*	*ml*
Control — no added sugar	1,701	302	372
Glucose	1,717	250	533
Fructose	1,757	275	534
Sucrose	1,766	276	557
Maltose	1,771	298	557
Mean	1,741	282	511
Standard error (single determination)	48.2	26.6	33.7
Coefficient of variability (%)	2.8	9.4	6.6

[a] 5% (flour basis).
[b] Means of duplicate determinations. Values are expressed at 0°C. and 760 mm. pressure.

Sample means (from duplicate determinations) which differed by more than twice the standard error of single determinations were considered significantly different.

The gas production by the sponges which were all of identical composition varied from 1,701 to 1,771 ml., with a coefficient of variability of 2.8% for the 4-hour fermentation period. This represents reasonably good replication for the instrument.

During the first 40 minutes of dough fermentation, corresponding to "floor time," the control dough with no added sugar produced significantly more gas than the doughs containing glucose, fructose, or sucrose. The dough containing 5% maltose produced significantly more gas than those containing glucose or fructose. This supports the previous observation based on sugar analyses, that, once yeast has become adapted to maltose fermentation in the sponge, it will still continue to utilize this sugar rapidly in the dough in the presence of glucose and fructose.

In the 60 minutes corresponding to the pan-proof period, all doughs containing added sugar produced significantly more gas than the no-sugar control. Those made with glucose, fructose, and sucrose all gave significantly more gas than the dough containing maltose. Maltose was fermented rapidly in the initial stages of dough fermentation, but the rate decreased with time during the pan-proof period.

Effect of Sponge Temperature on Sugar Levels in Fermenting Sponges and Doughs. The mean results of duplicate sugar determinations on sponges fermented at 25° and at 37°C., and on doughs fermented at 37°C. made from these sponges, are given in Table III.

The standard error of a single determination for the data in Table III is 0.05%, so that corresponding values could be considered significantly different from each other if they varied by 0.1% or more. Differences of this magnitude were found only in the glucose and fructose contents of doughs made with sucrose at the two temperatures, and they could have arisen as a result of the variations in yeast content of the formulas employed for the trials at the different temperatures. At both sponge temperatures, however, adaptation to rapid maltose fermentation apparently occurred.

Effect of Sponge Temperature on Gas Production in Sponges and

TABLE III

CHANGES IN SUGAR CONCENTRATION IN SPONGES FERMENTED AT 25° AND 37°C.[a] AND IN THE RESULTING DOUGHS FERMENTED AT 37°C. TO WHICH DIFFERENT SUGARS WERE ADDED

Mean sugar content (percent) after different fermentation times[b]

	Sugar Concentration								
	25°C. Sponge					37°C. Sponge			
	Fermentation (Minutes)					Fermentation (Minutes)			
	4	90	180	240	300	4	90	180	240
SPONGES									
Glucose	0.22	0.12	0.00	0.00	0.00	0.23	0.17	0.00	0.00
Fructose	0.30	0.29	0.00	0.00	0.00	0.28	0.25	0.00	0.00
Maltose	1.06	1.09	0.63	0.43	0.11	1.09	1.19	0.17	0.18
	Fermentation (Minutes)					Fermentation (Minutes)			
	4	40	100			4	40	100	
	no added sugar								
DOUGHS									
Glucose	0.05	0.04	0.00			0.05	0.04	0.00	
Fructose	0.07	0.08	0.00			0.13	0.13	0.00	
Maltose	0.63	0.52	0.32			0.56	0.41	0.33	
	5% glucose added[c]								
Glucose	2.70	2.17	2.05			2.77	2.38	2.10	
Fructose	0.07	0.11	0.12			0.07	0.11	0.11	
Maltose	0.68	0.58	0.51			0.75	0.58	0.54	
	5% sucrose added[c]								
Glucose	1.22	1.11	0.68			1.20	1.09	0.78	
Fructose	1.26	1.13	0.88			1.37	1.28	0.99	
Maltose	0.65	0.36	0.29			0.65	0.47	0.38	
	5% maltose added[c]								
Glucose	0.08	0.04	0.00			0.08	0.09	0.00	
Fructose	0.09	0.07	0.00			0.09	0.09	0.00	
Maltose	2.19	1.86	1.73			2.19	2.03	1.75	

[a] Doughs from sponges set at 25°C. contained 2.25% yeast; those from sponges set at 37°C. contained 2.0% yeast.

[b] Sugar concentrations are expressed as a percentage of the sponge or dough weight computed to a moisture content of 45.2%.

[c] Equivalent to 2.90% of dough weight.

Doughs. Zymotachygraphe gas-production curves for sponges fermented at 25° and 37°C. are given in Figs. 2 and 3. Also given are the curves for doughs with different added sugars, made from these sponges. In all cases the doughs were mixed and fermented at 37°C. The sugars added at the dough stage included 5% concentrations (flour basis) of glucose, fructose, sucrose, and maltose, as well as a no-sugar control. The curves for sucrose and fructose are omitted from Fig. 2 as they were essentially identical to that obtained with 5% added glucose.

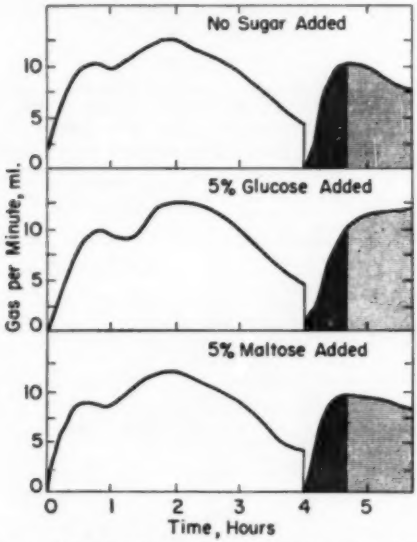

Fig. 2. Rate of gas production in sponges (0 to 4 hours) at 25°C. and in doughs (4 to 5.7 hours) containing 2.25% yeast (total flour basis), fermented at 37°C. The sugars were added at the dough stage. The cross-hatched and horizontally hatched areas represent the gas production in doughs during the first 40 and succeeding 60 minutes respectively.

In the sponge set at 25°C. the dip in gas production appeared after approximately 100 minutes, compared with about 60 minutes for the sponge set at 37°C. The sugar analyses reported in Table III show that at these times where gas production minima appeared, there were still appreciable concentrations of glucose and fructose present in both of these sponges.

The total gas production values for the sponges and doughs made from them are given in Table IV.

The sponge and dough formulas and fermentation times employed

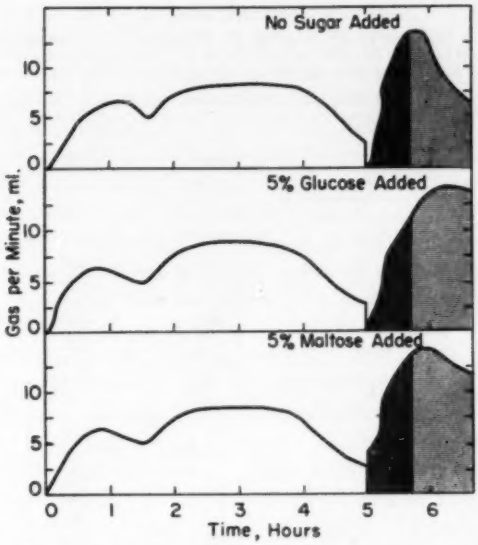

Fig. 3. Rate of gas production in sponges (0 to 4 hours) and in doughs (4 to 5.7 hours) containing 2.0% yeast (total flour basis), fermented at 37°C. The sugars were added at the dough stage. The cross-hatched and horizontally hatched areas represent the gas production in doughs during the first 40 and succeeding 60 minutes respectively.

at the two extremes of sponge temperature (25° and 37°C.) were based on those used commercially. The mean gas productions for all the sponges set at these temperatures were 1,600 and 1,888 ml. respectively. This significant difference in gas production indicates that the yeast levels and fermentation times were not adjusted to give sponges with the same degree of maturity. All doughs made from the sponges set at 25°C. produced considerably more gas during the pan-proof period than did corresponding doughs made from the sponges fermented at 37°C., especially when sugar was added to the dough.

The markedly lower gas production, during the pan-proof period, of the dough containing added maltose made from the sponge set at 37°C. than that of the corresponding dough made from the sponge fermented at 25°C. is of particular interest. The dough containing added maltose made from the 25°C. sponge yielded 159 ml. more gas during the pan-proof period than did the corresponding control dough. During this same 60-minute period the maltose level of the dough made with added maltose dropped from 1.86 to 1.73% (Table III). With the doughs made from the 37°C. sponges, gas production during

TABLE IV

GAS PRODUCTION IN SPONGES FERMENTED AT 25° AND 37°C. AND IN THE RESULTING DOUGHS CONTAINING DIFFERENT SUGARS WHICH WERE FERMENTED AT 37°C. FOR SUCCESSIVE INTERVALS OF 40 AND 60 MINUTES[a]

(Sponges set at 25°C. contained 2.25% yeast; those set at 37°C. contained 2.0% yeast)

SUGAR	GAS PRODUCTION[b]		
	Sponge	Dough	Pan-Proof
	ml	*ml*	*ml*
Sugar added[c]	25°C. 300 minutes	37°C. 40 minutes	37°C. 60 minutes
Control (no added sugar)	1,592	299	488
Glucose	1,550	254	704
Fructose	1,630	252	671
Sucrose	1,580	259	633
Maltose	1,648	292	647
Mean	1,600	271	629
Standard error (single determination)	52.3	24.1	15.4
Coefficient of Variability (%)	3.3	8.9	2.4
Sugar used in dough	37°C. 240 minutes	37°C. 40 minutes	37°C. 60 minutes
Control (no added sugar)	1,921	266	456
Glucose	1,803	228	576
Fructose	1,830	241	535
Sucrose	1,917	266	608
Maltose	1,917	248	475
Mean	1,888	250	530
Standard error (single determination)	47.5	11.2	16.6
Coefficient of Variability (%)	2.5	4.5	3.1

[a] Sponge weight taken for analysis equals 105 g. flour; dough weight equals 150 g. flour.
[b] Means of duplicate determinations. Volume corrected to 0°C. and 760 mm. mercury pressure.
[c] 5% (flour basis).

pan-proofing of the doughs made with and without maltose differed by only 19 ml., which is not statistically significant. The maltose level dropped from 2.03 to 1.75% in the 60-minute proof period for the dough containing 5% added maltose. The dough containing 5% maltose made from the 25°C. sponge produced 172 ml. more gas during the pan-proof period than did the corresponding dough made from the 37°C. sponge. That this difference in gas production is not paralleled by a corresponding difference in the rate of maltose utilization may be explained by the fact that the levels of maltose are the net result of production by the action of beta-amylase and of utilization by fermentation. Despite formula differences, it may be concluded that gas was produced more rapidly during the pan-proof period when doughs with 5% added maltose were made from sponges fermented at 25° rather than at 37°C. When sugars other than maltose were used the differences were not as great.

General Discussion

The results reported in this paper have shown that in the sponge

and dough process, if 5% glucose, fructose, or sucrose was added at the dough stage, there was adequate sugar to support rapid fermentation up to the time at which the dough entered the oven. In doughs containing 5% maltose or those made without any added sugar, gas production was more rapid in the initial stages of dough fermentation, but the rate decreased with time. It is possible that the relatively rapid initial rate of gas production in the doughs containing no sugar was due to a lower osmotic pressure than in the doughs containing 5% glucose, fructose, or sucrose. The addition of 5% maltose would not increase the osmotic pressure as much as would 5% glucose, fructose, or sucrose, as the molar concentration of maltose is only about half that of the glucose, fructose, or invert sugar (from sucrose).

The sponge and dough formulas and fermentation times employed for the two extremes of sponge temperature (25° and 37°C.) were based on those used commercially. The fact that the sponges set at 25°C. produced less gas during sponge fermentation than those set at 37°C., whereas all doughs made from the sponges at 25°C. produced more gas during the pan-proof period than those fermented at 37°C., indicates that the yeast levels and fermentation times were not well adjusted to the sponge temperatures. The "cool" (25°C.) sponges gave more rapid pan-proofing than did the "hot" (37°C.) sponges.

Acknowledgment

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STRUCTURE OF THE STARCH GRANULE
I. Swelling and Solubility Patterns of Various Starches[1]

HARRY W. LEACH,[2] L. D. MCCOWEN, AND THOMAS J. SCHOCH[2]

ABSTRACT

The modes of swelling of various starches have been evaluated over a range of pasting temperatures, to provide evidence of the associative bonding within the granule. The swelling pattern is greatly influenced by the species of starch. Thus corn and milo starches show a limited two-stage swelling, indicative of two sets of bonding forces which relax at different temperatures. In contrast, potato starch undergoes very rapid and unrestricted swelling at relatively low temperature, indicating weak and uniform bonding. In general, the extent of starch solubilization during pasting parallels the swelling pattern. These two techniques have been used to study the architecture within the granule, and the influence of such factors as content of linear and branched fractions, heat-moisture treatment, presence of fatty adjuncts, and chemical modification of the starch.

There has been considerable speculation on the nature of the molecular architecture within the starch granule, but without much experimental evidence. K. H. Meyer (2) formulated the concept of granule structure which is most generally accepted. According to this theory, the intermixed linear and branched starch molecules are arranged in the granule in a radial fashion (Fig. 1,A). Wherever linear segments of either branched or linear molecules parallel one another, hydrogen bonding forces pull the chains together into associated crystalline bundles or "micelles." A long linear chain may conceivably pass through a number of such micellar areas, or the outer fringe branches of a branched molecule may participate in several separate micelles. Hence these crystalline areas act to hold the granule together, to permit swelling but prevent dispersion and solution of the individual starch molecules. In the spaces between the micelles, the chains and branches are more disordered and hence less densely packed. When an aqueous suspension of granular starch is heated, it is presumed that these more amorphous areas undergo progressive hydration and swelling. Eventually, this gives the expanded network shown in Fig. 1,B, held together by the persistent and intact micelles. It is this network which gives the swollen granule its elastic character and which is responsible for most of the viscous qualities of a cooked starch paste. The micellar areas can only be dispersed by such means as autoclaving.

While this theory has been very useful, it still leaves certain ques-

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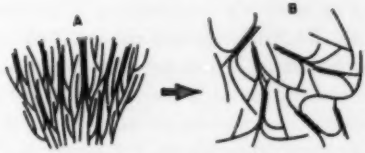

Fig. 1. Swelling of a segment of the starch granule according to K. H. Meyer. A, unswollen network; B, swollen network. Thickened sections indicate micelles.

tions unanswered, e.g., the differences in behavior of corn and potato starches, and the roles of the linear and branched fractions in granule architecture. In the present work, it was postulated that the bonding forces within the starch granule would influence its manner of swelling. Thus a highly associated starch (i.e., with an extensive and strongly-bonded micellar structure) should be relatively resistant toward swelling. If the extent of swelling is plotted against temperature of pasting, a curve should be obtained which represents progressive relaxation of the bonding forces within the granule. This would permit comparison of the relative bond strengths in various starches from the temperature (i.e., energy level) necessary to cause relaxation.

Methods

Granule swelling is determined over the pasting range by the following procedure. An appropriate amount of the starch (usually 1–10 g.) is suspended in 180 ml. of distilled water in a tared 250-ml. centrifuge bottle. The suspension is mechanically stirred with a small stainless-steel paddle (0.75-in. wide, 1.5-in. high) at a rate just sufficient to keep the starch completely suspended (i.e., 200 r.p.m.) This low speed avoids shearing the fragile swollen granules and consequent solubilization of the starch. The bottle is lowered into a thermostatted water bath maintained at the desired temperature (± 0.1°C.) and held for 30 minutes, slow stirring being continued during this period. The bottle is then removed, wiped dry, and placed on the torsion balance. The stirrer is removed and rinsed into the bottle with sufficient distilled water to bring the total weight of water present to 200.0 g. (including the moisture in the original starch). The bottle is stoppered, mixed by gentle shaking, and then centrifuged for 15 minutes at 2200 r.p.m. (i.e., 700 times gravity). The clear supernate is carefully drawn off by suction to within ¼ in. of the precipitated paste. An aliquot of this supernate is evaporated to dryness on the steam bath and then dried for 4 hours in the vacuum oven at 120°C. The percentage of solubles extracted from the starch is calculated to dry basis. The remaining aqueous layer above the sedimented starch paste is then

siphoned off as quantitatively as possible. The bottle and paste are reweighed on the torsion balance, and the swelling power calculated as the weight of sedimented paste per g. of dry-basis starch. This value is corrected for solubles to provide a measure of swell of the undissolved portion of the starch. Determinations of swelling power and solubles were run at temperature intervals of 5°C. over the swelling range of the starch, i.e., from the point of initial gelatinization to 95°C.

Precision of results is remarkably good. At swelling powers less than 25-fold, average deviation from the mean is ± 0.16 unit for swelling power and ± 0.13% for solubles. At higher swelling powers, precision values are ± 0.9 unit and ± 0.5%, respectively. Potato starch showed wider deviations, owing to its very high swelling power and the difficulty in obtaining a clean-cut separation of the supernatant solution.

Results and Discussion

Effects of Starch Species. The patterns of swelling and solubilization are greatly influenced by the species of starch. As shown in Fig. 2, potato starch undergoes a very rapid and almost unrestricted swelling at relatively low temperature. This indicates weak bonding forces of approximately uniform strength. In comparison, tapioca starch commences to swell at almost the same temperature as potato starch, but the swelling thereafter proceeds at a much slower rate. Hence it is presumed that the associative forces within the tapioca granule represent a much wider range of bond strengths than those in po-

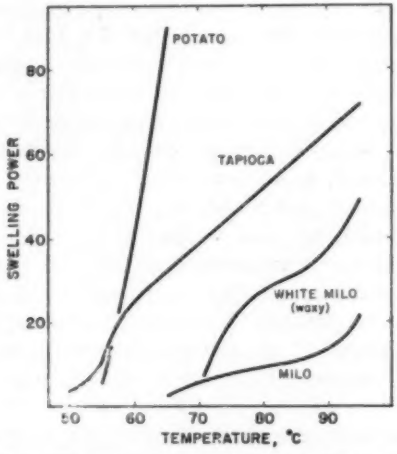

Fig. 2. Swelling patterns of various unmodified starches.

tato starch. As an analogy, the two starches might be likened to two liquids, having a narrow and a wide distillation range, respectively. Another type is exemplified by the two-stage swelling of white milo (waxy sorghum) and regular milo starches.[3] These starches show initial gelatinization, then a period of restricted swelling, and finally a second rapid swelling. This behavior is attributed to two sets of bonding forces which relax at different temperature levels.

When solubilities of these four starches are plotted against pasting temperature, the resulting curves (Fig. 3) are very similar to their swelling patterns. Here again, milo and waxy white milo starches show

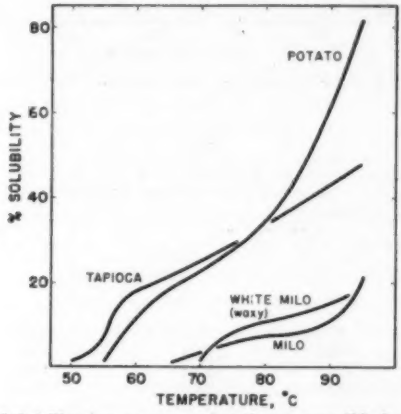

Fig. 3. Solubilization patterns of various unmodified starches.

pronounced two-stage solubilization, indicative of two sets of bonding forces within the granules. Plotting the swelling power against percentage solubles (Fig. 4) provides an interesting comparison of the solubilization of these starches at equal levels of swelling. Despite its high and seemingly unrestricted swelling, potato starch is much less soluble at any particular degree of swell than are the other starches. Hence, while the bonding forces in the potato starch granule are very weak and tenuous, nevertheless they are comparatively extensive, immobilizing the starch substance within the granule even at very high levels of swelling. As shown in another connection in Table I, unmodified potato starch swells several hundredfold before extensive solubilization occurs.

[3] Note added June 19, 1959. A previous publication (1) from this laboratory showed somewhat higher swelling power and solubility for white milo starch. Subsequent manufacturing improvements have given a starch of higher hot-paste viscosity, lower swelling power, and lower solubility. The present data were obtained on three batches representative of recent production.

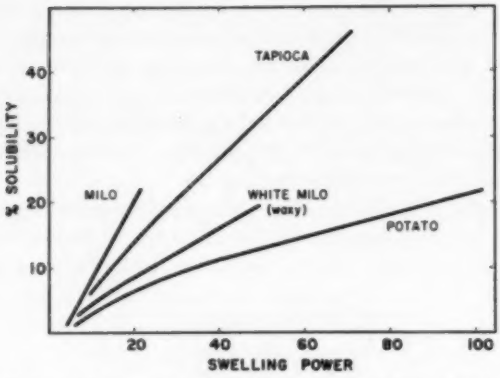

Fig. 4. Relationship between swelling power and solubility of various unmodified starches.

TABLE I

EFFECT OF HEAT-MOISTURE TREATMENT ON THE SWELLING POWER AND SOLUBILITY OF POTATO STARCH

PASTING TEMPERATURE	SWELLING POWER		SOLUBILITY	
	Untreated	Treated	Untreated	Treated
°C				
55	5.9	...	1.1	....
65	87.0	3.8	18.5	0.41
75	160.0	7.0	28.0	2.1
85	372.0	13.4	45.0	6.9
95	1153.0	23.0	82.0	17.9

The swelling and solubility curves of corn and wheat starches resemble those of milo starch, while arrowroot and waxy maize starches are similar to the waxy white milo. To show the influence of the linear fraction, swelling and solubility curves were obtained on wrinkled pea starch (variety Laxton's Progress) and on a high-linear corn starch (phenotype aeae):

Starch	*Percent Iodine Affinity*	*Swelling Power at 95°C.*	*Percent Solubility at 95°C.*
Wrinkled pea	15.0	5.5	19.1
High-linear corn	11.9	6.3	12.4

Both starches showed greatly restricted single-stage swelling and solubility. Even after 30 minutes at 95°C., the pastes still contained a substantial number of birefringent ungelatinized granules. The swelling curves and particularly the solubilities indicated a greater degree of association in the high-linear corn starch than in the wrinkled pea

starch, despite the higher linear content of the latter.

Floury Endosperm Starch. Corn starch separated from the floury endosperm shows the same swelling pattern as commercial corn starch, but has a significantly lower solubility. This effect was observed with a corn starch sample obtained at an intermediate stage in the factory wet-milling process, that is, after germ separation but before buhr milling to release the starch from the horny endosperm. A commercial German corn starch from South African white corn likewise showed lower solubility, presumably due to a higher proportion of floury endosperm in the parent corn. The starch from Cuzco floury corn (Peru) also showed normal swelling but reduced solubility.

Effect of Drying. The mode of drying corn starch does not alter its swelling pattern. Thus identical swelling curves were obtained on the following samples: wet starch cake from the factory filters, wet starch dried at room temperature, starch dried in the countercurrent Proctor-Schwartz dryer, kiln-dried corn starch, and flash-dried corn starch. Unfortunately, solubility curves were not obtained on these five samples. Starch was also prepared from dent corn picked at a late "dough" stage of field-drying (i.e., the shelled kernels contained 33–36% moisture). Subsequent tests were run on the wet starch cake, without drying. This product had the same Kofler gelatinization temperature, Brabender viscosity, and swelling pattern as commercial corn starch. However, it showed lower solubility because the mode of preparation eliminated much of the horny endosperm. In general, these results indicate that the conventional factory drying processes do not significantly alter the qualities of the native corn starch.

Effect of Aging the Starch Paste. It was of practical interest to determine the dimensional stability of the swollen granule on standing and reheating. When corn starch is pasted at temperatures in the range of 60°–95°C., then cooled and allowed to age for 24 hours at room temperature, the swollen granules do not undergo any shrinkage but maintain the same volume attained during pasting. If these aged pastes are reheated to the original pasting temperature, the volume of the swollen granules remains constant. If the aged pastes are reheated higher than the original pasting temperature, the granules undergo some additional swelling, but not an amount commensurate with the higher temperature. These latter results indicate that some retrogradation probably occurs within the swollen granule during aging of the paste, which stabilizes the granule against swelling when heated to a higher temperature.

Heat-Moisture Treatment. Sair and Fetzer (3) have shown that the physical character of potato starch may be radically altered by heating

the granular starch for extended periods of time at high humidity levels. According to their procedure, the starch was preadjusted to a moisture level of 20–25%, then heated for several hours in closed tubes at 100°C. Microscopic examination showed no change in granule appearance or optical polarization of the treated starch. However, this heat-moisture treatment changed the X-ray diffraction spectrum of the potato starch from its normal B-pattern to an A-pattern similar to that of corn starch. In addition, the treated starch behaved more like corn starch in several other respects, having a lower equilibrium moisture level, and giving "short" opaque pastes which set up to rigid gels on cooling. Hence it is presumed that the heat-moisture treatment has caused the starch substance to recrystallize in a molecular lattice similar to that of corn starch.

As a more convenient method of heat-moisture treatment, the granular starch may be heated in a mixture of diacetone alcohol and water. The proportion of water in the mixture is carefully adjusted to permit hydration of the starch but avoid gelatinization. For example, one part of potato starch is suspended in three parts of 69% diacetone alcohol, i.e., adjusted to contain 31% by weight of water, including the moisture in the starch. This mixture is gently boiled under reflux for 4 hours, with stirring meanwhile to maintain uniform suspension. The starch is then filtered off, thoroughly washed with distilled water, and air-dried. Microscopic examination shows no gelatinized granules as judged by loss of polarization crosses. However, a minor proportion of the granules may show a small dark spot at the hilum when mounted in clove oil, possibly indicative of either cavitation or incipient gelatinization in this region. The Kofler gelatinization temperature of the treated starch is raised some 5° to 10°C.

As shown in abbreviated form in Table I, both swelling power and solubles are greatly reduced by heat-moisture treatment. This is attributed to an increase in the strength of associative bonding within the granule. However, one alternative possibility had to be considered. Potato starch contains a significant amount of esterified phosphate which might cross-bond the molecules during heat-moisture treatment. This would radically reduce both swelling power and solubility of the starch (1). As evidence against such chemical cross-bonding, tapioca starch (which contains very little phosphorus) likewise shows a great decrease in both swelling power and solubility after heat-moisture treatment. In addition, waxy maize and waxy white milo starches (which contain moderate levels of phosphorus) are not significantly altered by heat-moisture treatment.

Theory of Granule Structure. It seems plausible that the granule

structures of both milo and waxy white milo starches consist of micellar regions scattered through an amorphous matrix, as shown in Fig. 1,A. Initial swelling probably occurs in the amorphous areas and is not affected by the presence of micelles. Hence the gelatinization temperature of waxy white milo is the same as that of regular milo (namely, 67.5°–74°C.), and waxy maize is the same as corn (namely, 62°–72°C.). The higher gelatinization temperature of the milo starches may indicate a higher degree of association in the amorphous areas. The presence of a network of linear molecules interconnecting the micelles does not ordinarily affect the gelatinization temperature, but does restrict subsequent swelling of the granule. This would explain the limited swelling of the cereal starches as compared to their waxy counterparts. Only a portion of the linear fraction of corn starch is involved in the micellar structure. Thus, previous studies (4) have shown that approximately half of the total linear fraction is readily leached from the swollen corn starch granule. This consists of short-chain linear material which presumably originated from the amorphous areas. In exceptional cases (namely, high-linear corn and wrinkled pea starches), most of the granule is highly organized and there are no "soft" amorphous regions to undergo swelling. Such granules may show no gelatinization even at 95°C.

Potato starch presents a very different pattern of behavior which cannot adequately be explained by the granule organization in Fig. 1,A. It swells much more freely than even the waxy starches, which suggests the absence of any micellar organization. Yet at any particular level of swelling, potato starch gives less solubles than the cereal starches. In addition, the linear fraction is much more difficult to leach from the swollen potato starch granule (i.e., only 20% of the total linear fraction is dissolved, at a temperature just above the gelatinization temperature, as compared with 50–60% for corn starch). Hence it appears that the linear potato starch fraction is more intimately entangled in the granule structure. The tenuous molecular network of potato starch may be considered as more extensive and uniform than that of the cereal starches, with no areas of strong micellar association but likewise no areas of random disorganization. Since the equilibrium moisture of potato starch is substantially higher than that of the cereal starches, it is conceivable that hydrogen bonding occurs through hydrate water bridges rather than by strong direct association of linear and branched molecules. Heat-moisture treatment may dissociate this hydrate water and induce rearrangement to a strong micellar pattern interconnected by linear molecules, similar to that depicted in Fig. 1,A.

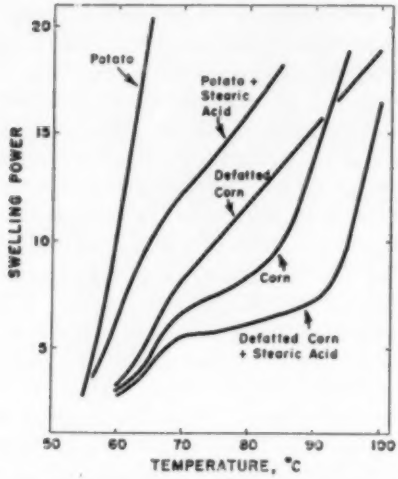

Fig. 5. Effect of fatty acid on swelling of corn and potato starches.

Effects of Fatty Adjuncts. It is well known that the higher fatty acids inhibit granule swelling, probably by forming insoluble complexes with the linear fraction. As shown in Fig. 5, defatted corn starch swells freely and uniformly, with little evidence of the two-stage pattern of unmodified corn starch. To confirm this effect by restoration of fatty acid, 250 g. of defatted corn starch were suspended in water, a solution of 6 g. of sodium stearate was added, and the mixture stirred for 30 minutes. The starch was then thoroughly washed with distilled water to remove excess soap, adjusted to pH 5.0 with acid, filtered, washed, and air-dried. This product showed even greater restriction of swelling than the original corn starch. Potato starch similarly impregnated with stearic acid likewise showed decreased swelling. The swelling of corn starch in the presence of polyoxyethylene monostearate was very similar to the pattern of corn starch impregnated with stearic acid. However, such surfactants as sodium lauryl sulfate (DuPont's "Duponol" ME) or octadecyltrimethyl ammonium chloride (Armour's "Arquad" 18) did not significantly alter granule swelling. Presence of sodium stearate caused a rapid single-stage swelling of corn starch in the range of 65°–75°C.; however, this action is attributed entirely to high pH (8.5) and not to the stearate.

Effect of Chemical Modification. When granular starch is treated with warm dilute acid, hydrolysis presumably occurs in the more accessible intermicellar areas, thereby weakening the network within the

granule. Consequently, when these so-called thin-boiling starches are cooked in hot water, the swollen granule falls apart to give pastes of reduced viscosity. This theory is borne out by the greatly increased

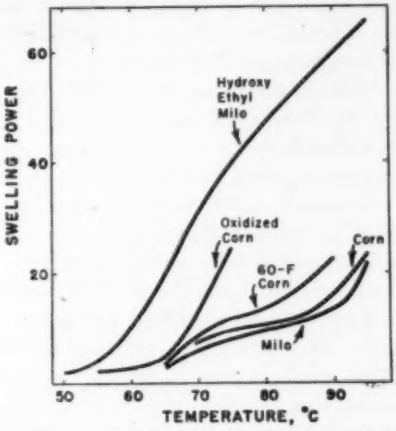

Fig. 6. Swelling patterns of unmodified milo starch, unmodified corn starch, 60-fluidity acid-modified corn starch, thin-boiling hypochlorite-oxidized corn starch, and thick-boiling hydroxyethyl milo starch (0.1 D.S.).

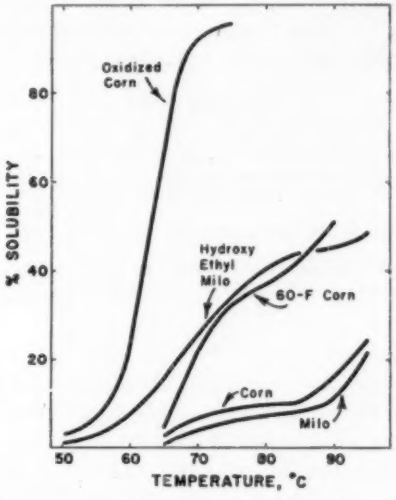

Fig. 7. Solubilization patterns of unmodified milo starch, unmodified corn starch, 60-fluidity acid-modified corn starch, thin-boiling hypochlorite-oxidized corn starch, and thick-boiling hydroxyethyl milo starch (0.1 D.S.).

swelling and solubility of a commercial 60-fluidity corn starch (Figs. 6 and 7). A comparable 60-fluidity waxy white milo starch showed even weaker granule structure, since this product gave over 90% solubles when pasted at 80°C. In this instance, there was no connecting reinforcement of linear molecules to hold the granule together.

Hypochlorite oxidation is likewise a highly effective means for weakening the internal structure of the granule. As depicted in Figs. 6 and 7, a commercial oxidized corn starch (100-g. Scott viscosity = 56) literally fell apart and dissolved without swelling. In contrast, a thick-boiling hydroxyethyl milo starch of 0.1 D.S. (i.e., "degree of substitution" = one hydroxyethyl group per ten glucose units) showed a slow uniform swelling over the entire pasting range of 55°–95°C. This might indicate that hydroxyethylation of granular starch occurs primarily in the more accessible intermicellar areas, and hence sufficient micellar structure persists to hold the granule together. As corroborative evidence, corn starch was gelatinized in aqueous alkali to open up the micellar structure, then etherified with ethylene oxide to the 0.25-D.S. level. This product gave a red color with iodine (iodine affinity = 0.0%), indicating that the linear fraction had been uniformly derivatized. In contrast, granular corn starch was reacted with ethylene oxide under conditions which did not cause gelatinization, to give an ether likewise of 0.25 D.S. This product gave a strong blue color with iodine (iodine affinity = 1.62%). In the latter instance, it is presumed that one-third of the linear fraction was involved in micellar organization and hence inaccessible to the etherifying agent.

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THE EFFECT OF LYSINE SUPPLEMENTATION ON THE PROTEIN EFFICIENCY OF HIGH-PROTEIN BREADS[1]

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ABSTRACT

Rat-growth studies were carried out with diets in which a commercial white bread and three representative types of commercial protein bread were the sole sources of protein, with and without lysine supplementation. The percentage of protein from wheat varied from 89 to 60%, that from milk and soy proteins from 11 to 40%. Over this range of compositions, the protein quality as measured by the protein efficiency ratio was found to be a direct function of the lysine content of the mixed protein, regardless of whether the lysine was a constituent of protein or was added as L-lysine monohydrochloride. For protein efficiency ratios in the high-quality range of 2 or higher, it appears necessary that the bread protein contain at least 200 mg. of L-lysine per gram of nitrogen.

The investigations of Rosenberg and Rohdenburg (10), Hutchinson, Moran, and Pace (6), and Jahnke and Schuck (7) demonstrated that the addition of 0.25 part L-lysine monohydrochloride per 100 parts flour almost doubled the protein efficiency ratio of white bread baked from this flour. The breads tested by the first two groups of investigators contained 0 and 3 parts nonfat dry milk per 100 parts flour, and it is obvious that nearly all of the protein in these breads was derived from wheat. However, Jahnke and Schuck tested diets simulating bread formulations containing as much as 12 parts nonfat dry milk, so that in this case the total dietary protein was derived in significant measure from high-quality milk protein. Yet the protein efficiency ratio of this mixture was found to be markedly increased by the addition of free lysine. Since many of the new "protein" breads may contain proportions of milk or other good-quality protein even greater than those tested by Jahnke and Schuck, it was of interest to determine whether the protein efficiency ratio of such foods could likewise be increased by lysine supplementation and also whether their protein efficiency ratio had any direct relation to their lysine content.

Methods

Albino rats of the Charles River strain were received in the laboratory at 21 days of age (approximately weight, 40 g.) and immediately started on their assigned diets. Each diet was fed to 20 animals, 10 males and 10 females. They were individually housed with food

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and water available *ad lib.* Body weight and food consumption were measured at weekly intervals throughout the feeding period, which was 35 days for the male rats and 36 days for the female rats.

A commercially available white bread and three different brands of protein bread were tested. The breads were dried by laying the slices on paper spread on the laboratory floor. For most, a single overnight exposure was sufficient to reduce moisture content to about 8%. All of the breads were analyzed for water, nitrogen, and lysine content. Moisture was determined by drying to constant weight at 105°C., nitrogen was determined by micro-Kjeldahl analysis, and lysine was estimated by a standard microbiological assay procedure (Steele *et al.*, 12). The dried bread was passed through a standard coffee mill before it was incorporated into the diets. Both the crust and crumb of the bread were included in the diets.

The composition of the diets is summarized in Table I. By the addition of corn oil and sucrose in varying amounts, all diets were made to contain about 8% fat and 70–80% carbohydrate. From analysis of the finished diets it was determined that the rations contained 1.93–2.04% nitrogen. Protein contents, varying from 11.1 to 13%, were calculated by multiplying the nitrogen contents by the appropriate factors for the particular sources of protein (Orr and Watt, 9). The

TABLE I

COMPOSITION OF DIETS

Source of Protein	Percent by Weight, Basic Food in Diet	Other Nutrients Added Percent by Weight of Diet, Dry Weight Basis				Percent of Nutrient in Diet, Dry Weight Basis[c]		
		Corn Oil	Sucrose	Salts[a]	Vitamins[b]	Protein[d]	Fat	Carbohydrate
White bread[e]	76	4	16	3	0.73	11.1	7.82	77.0
Gluten protein bread	42	7	47	3	0.73	11.1	7.86	79.5
Soy-milk protein bread	67	7	22	3	0.73	11.7	8.37	73.6
Milk-lactalbumin-soy protein bread	55	7	34	3	0.73	11.8	8.09	77.5
Casein (control)[f]	13	8	20	3	1.0	13.0	8.58	68.6

[a] Salt Mixture No. 2, USP XIII: Calcium biphosphate, 13.58%; calcium lactate, 32.70%; ferric citrate, 2.97%; magnesium sulfate, 13.70%; potassium phosphate (dibasic), 23.98%; sodium biphosphate, 8.72%; sodium chloride, 4.35%.

[b] Approximately 27% of the vitamin space was used for the lysine and glycine additives. The designed vitamin content (at 0.73% of the diet) was computed from the manufacturer's data to be, in mg. per 100 g. diet: vitamin A concentrate (200,000 units per g.), 7.2; vitamin D concentrate (400,000 units per g.), 0.40; alpha tocopherol, 8.0; ascorbic acid, 72.2; inositol, 8.0; choline chloride, 120.5; menadione, 3.6; *p*-aminobensoic acid, 8.0; niacin, 7.2; riboflavin, 1.6; pyridoxine hydrochloride, 1.6; thiamine hydrochloride, 1.6; calcium pantothenate, 4.8; and in micrograms per 100 g. diet, biotin, 32.1; folic acid, 145.3; and vitamin B-12, 2.2.

[c] Ground bread approximately 8% moisture. Fat and carbohydrate content calculated from data supplied by the commercial baker.

[d] Protein content of the diets was calculated from the nitrogen content, using the following factors: soy, wheat gluten, and white flour protein, N × 5.7; yeast protein, N × 6.25; milk proteins, N × 6.4; (Orr and Watt, 9).

[e] Contained 5 g. nonfat dry milk per 100 g. flour.

[f] "Vitamin-free" casein from Nutritional Biochemicals Corporation, Cleveland, Ohio. Remainder of casein diet: 50% corn starch, 5% cellulose.

energy content of the diets was estimated to be about 420 calories per 100 g. A 13% casein diet similar in composition to the bread diets served as a control. The carbohydrate in the casein diet consisted of corn starch, sucrose, and cellulose (50, 20, and 5% of the diet, respectively). Lysine was added to the diets as L-lysine monohydrochloride[4] in the ratio of 0.25 g. per 12 g. wheat protein. The proportion of the total protein that was derived from wheat was calculated from recipe data supplied by the baker. In the bread diets to which a lysine supplement was not added, an isonitrogenous amount of glycine was supplied.

At the end of the feeding period, three male and three female rats from each diet group were sacrificed, and the livers of these animals were analyzed for fat, nitrogen, and moisture content. Moisture and nitrogen were determined as noted above. Fat was determined as the weight of dry residue from a diethyl ether extract of dried liver.

Results

Differences in appearance of the animals on lysine supplementation from those on the isonitrogenous glycine supplement were apparent almost from the beginning. The fur of the animals receiving the lysine-deficient diets was rough and lusterless, although the difference tended to be reduced when the diet contained substantial amounts of milk or soy protein. The ears and paws of the animals on the glycine supplement were frequently pale, and there was usually an obvious difference

TABLE II

EFFECT OF LYSINE SUPPLEMENTATION ON RAT GROWTH AND THE PROTEIN EFFICIENCY OF BREAD DIETS

Source of Protein	Wheat Protein As Percent of Total Protein[a]	LMH[b] Added, Percent of Diet	Rat Sex	Average Weight Gain for 5 Weeks		Average PER[d]	
				Control[c]	LMH Added	Control[c]	LMH Added
				g	g		
White bread	86	0.20	M	25	84	1.14	2.15
			F	30	74		
Gluten protein bread	89	0.22	M	22	72	0.94	2.03
			F	21	75		
Soy-milk protein bread	70	0.15	M	54	98	1.62	2.47
			F	57	100		
Milk-lactalbumin-soy protein bread	60	0.15	M	60	107	1.89	2.57
			F	72	114		
Casein (control)	...	...	M	65	...	1.92	
			F	60			

[a] Calculated from data supplied by commercial baker.
[b] LMH is L-lysine monohydrochloride (Du Pont product containing 95% L- and 5% D-isomer).
[c] Sufficient glycine added to make the diet isonitrogenous with the corresponding lysine-supplemented diet.
[d] PER is protein efficiency ratio, defined as g. weight gained divided by g. protein consumed.

[4] Du Pont L-lysine monohydrochloride, containing 95% L- and 5% D-isomer.

in body size. Also, the glycine-supplemented animals tended to huddle in the corner of the cage. Their activity was markedly reduced, and the animals were definitely more irritable than their counterparts on the lysine supplement.

The data presented in Table II summarize the effect of lysine supplementation on rat growth and the protein efficiency of the various bread diets. It will be noted that the level of lysine supplementation varied, depending on the proportion of total protein supplied by wheat. In general, good correlation was noted between body weight gain and the protein efficiency ratio (PER)[5] of the diet. The PER of the unsupplemented white bread diet (five parts nonfat dry milk) was approximately 1.1 and was increased to more than 2 by supplementation with lysine. This confirmed the results of previous studies on white bread (Rosenberg and Rohdenburg, 10; Hutchinson *et al.*, 6; Jahnke, Schuck, 7). The PER of the diet containing the gluten protein bread without added lysine was somewhat lower than that of the white bread diet, possibly because of the higher proportion of lysine-deficient wheat protein, or because of the difference in sucrose and starch contents. However, lysine supplementation again brought the efficiency ratio to 2. The PER's of the unsupplemented soy-milk and milk-lactalbumin-soy protein breads were significantly above that of ordinary white bread or the unsupplemented gluten protein bread. Thus, the addition of good-quality protein not only increased the protein quantity but also the protein quality of these breads as compared with standard white bread. In these cases good-quality protein accounted for 30–40% of the total, the remainder being white flour protein. The protein efficiency of the soy-milk bread was found to be 1.6, and that of the milk-lactalbumin-soy bread about 1.9. However, supplementary lysine further increased the protein quality of both breads, bringing the PER of the soy-milk bread to almost 2.5, a 50% improvement, and that of the milk-lactalbumin-soy bread to about 2.6, a 36% increase.

The observed protein efficiency ratios are not necessarily the maximum ones that could be obtained with these breads. No attempt was made to explore the further improvements that might be obtained by adding other amino acids or higher levels of lysine.

The lysine and glycine supplements used in these studies were added to the bread ingredients of the diets after, rather than before, baking. Somewhat higher levels of lysine supplementation might have to be used to obtain protein efficiency ratios equivalent to those reported here if the amino acid were added before baking.

[5] Defined as the ratio: g. of weight gained during the experimental period divided by g. of protein consumed.

The work of Deshpande and co-workers (4) and Elvehjem (5) has brought into focus the striking effect of amino acid balance in low-protein diets on liver fat content. In the present study, therefore, three male and three female rats from each diet group were sacrificed and their livers analyzed for fat, protein, and moisture content. The results of these determinations are summarized in Table III. It would appear that the lysine supplementation levels used in the present study had no significant effect on these values. However, it is interesting that the livers of rats on the casein diet contained somewhat less fat and more protein than the livers of animals on the bread diets, and lysine supplementation of the bread diets did not change this relation.

TABLE III

FAT AND PROTEIN CONTENTS OF LIVER OF RATS ON LYSINE-SUPPLEMENTED AND UNSUPPLEMENTED BREAD DIETS

SOURCE OF PROTEIN	L-LYSINE HYDROCHLORIDE	RAT SEX	FAT[a]	PROTEIN[a]
			% dry wt.	*% dry wt.*
White bread	None	M	19.6	48.2
	None	F	17.9	46.8
	Added	M	15.1	51.4
	Added	F	16.8	42.5
Gluten protein bread	None	M	16.8	46.6
	None	F	14.3	46.4
	Added	M	14.9	47.4
	Added	F	21.3	55.0
Soy-milk protein bread	None	M	15.6	46.6
	None	F	18.9	46.4
	Added	M	15.6	47.4
	Added	F	18.6	55.0
Milk-lactalbumin-soy protein bread	None	M	16.1	42.1
	None	F	15.7	51.2
	Added	M	13.0	47.6
	Added	F	13.0	58.6
Casein (control)	.....	M	5.46	58.7
	.....	F	9.35	68.1

[a] Average of three determinations. Protein calculated as N × 6.25.

The "vitamin-free" grade of casein employed in the control diet appeared to have been somewhat damaged in nutritional value or palatability by its processing, and no significance should be attached to a comparison between the bread diets and the control diet in total gain or protein efficiency ratio. However, the casein-fed animals were normal in appearance and activity and, as just noted, had livers higher in protein and lower in fat than the bread-fed animals.

The lysine content of the various breads was determined by microbiological assay, and the lysine content of the diets calculated using these values. The lysine and nitrogen contents are tabulated in Table

IV. To determine whether or not the supplementary lysine was being utilized for growth by the animal in a manner similar to its utilization

TABLE IV

LYSINE AND NITROGEN CONTENTS OF BREAD DIETS

SOURCE OF PROTEIN	LYSINE IN BREAD[a]	BREAD IN DIET	LYSINE IN DIET[b]		NITROGEN IN DIET[c]
			None Added	Lysine Added	
	%	%	%	%	%
White bread	0.32	76	0.24	0.40	1.94
Gluten protein bread	0.53	42	0.22	0.40	1.93
Soy-milk protein bread	0.47	67	0.31	0.44	2.03
Milk-lactalbumin-soy protein bread	0.73	55	0.40	0.52	2.00

[a] Bread contained 8% moisture.
[b] Calculated as free L-Lysine, not as the monohydrochloride.
[c] Diets with no lysine added were made isonitrogenous by addition of glycine.

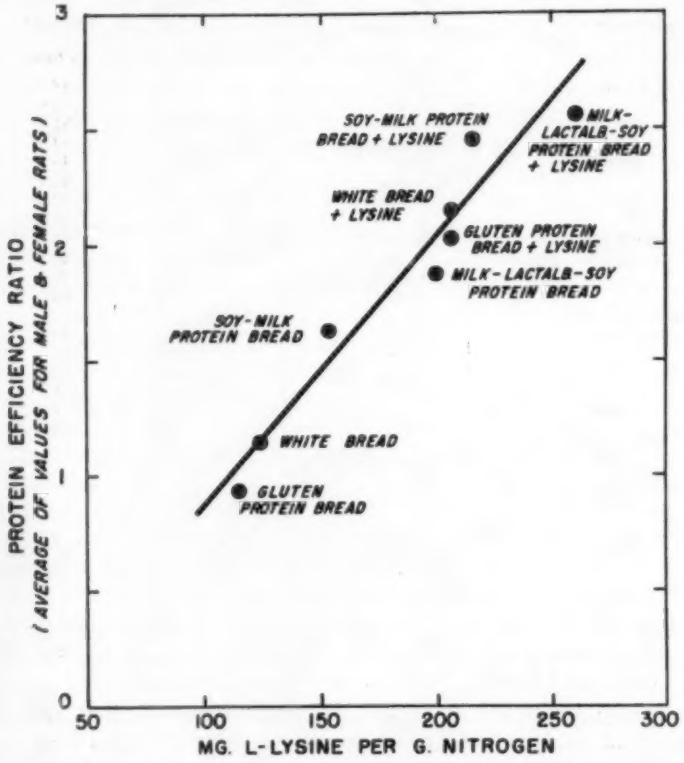

Fig. 1. Effect of lysine content of bread diets on protein efficiency.

of lysine from food protein, the lysine content of the proteins in the supplemented and unsupplemented diets (as mg. L-lysine per g. of nitrogen) was plotted against their protein efficiency ratios. From the linear relation shown in Fig. 1, it appears that the growth of the animals is stimulated to the same extent by increasing the proportion of lysine in the dietary protein, by the addition of either high-quality protein or L-lysine monohydrochloride.

Discussion

The diets used in this study provided about 11% of their calories in the form of protein, a level high enough to permit good growth if the amino acid balance is efficient. With the diets whose balance was defective by virtue of a relatively low proportion of lysine, symptoms were produced that were similar to those observed in children suffering from protein deficiency. These symptoms included, besides growth retardation, changes in appearance of the hair, irritability, and reduced activity. Similar signs are noted in kwashiorkor (Scrimshaw *et al.*, 11). Retarded growth, lack-luster hair, and apathy have also been reported as occurring frequently among children in Newfoundland, where white flour is a major source of dietary protein (Metcoff *et al.*, 8; Aykroyd *et al.*, 3). Aykroyd *et al.* noted a great reduction in prevalence of lack-luster hair and apathy among Newfoundland children in 1948, following four years of flour enrichment, as compared with findings in 1944. They ascribed this improvement to increased thiamine intakes, but it should be noted that some improvement in intake of high-quality protein occurred at the same time, for example through distribution of a milk drink to school children beginning in 1946-1947. The reduction in physical activity may reflect a depleted state of labile protein reserves, or, specifically, of enzyme systems involved in the conversion of calories to physical work. Allison has demonstrated in dogs that high-quality proteins are much more effective than low-quality proteins in maintaining adequate labile reserves (1), and that puppies fed wheat gluten are less active than those grown on high-quality proteins (2).

Since white flour used in the baking of bread usually contains about 12% protein, a supplementation level of 0.25 parts L-lysine monohydrochloride per 100 parts flour corresponds to 0.25 part of the amino acid salt per 12 parts wheat protein. The diets used in the present study also contained close to 12% protein, and if all of the protein had been derived from wheat, the diets would have been made to contain about 0.25% added L-lysine monohydrochloride. However, the breads tested derived varying proportions of their total protein from milk and soy, proteins which may be presumed to be adequate in lysine. Thus,

the lysine supplementation levels were adjusted so that only the wheat portion of the total protein was supplemented at the above-mentioned rate. For example (see Table II), it was calculated from data supplied by the baker that about 89% of the total protein in the gluten protein bread was derived from wheat. Thus, the supplementation level used was 0.22% (0.89× 0.25) of the diet. On the other hand, the milk-lactalbumin-soy protein bread derived only about 60% of its total protein from wheat, and the L-lysine monohydrochloride supplementation level was dropped, therefore, to 0.15% of the diet. Even this low level of lysine supplementation of a diet deriving as high as 40% of its protein from soy and milk increased the protein efficiency ratio of the dietary protein by 36%.

It was hypothesized, from the amino acid composition of the various proteins in the diets studied, including those supplied by white flour, wheat gluten, nonfat dry milk, lactalbumin, soy flour, and baker's yeast, that lysine would be the first limiting amino acid in the mixed protein of these diets. Assuming the hypothesis to be true, one should observe an increase in the protein efficiency in direct relation to an increase in the content of lysine in the mixed protein. The validity of this assumption is indicated by Fig. 1, which, over the range of compositions studied, shows a linear relation between the protein efficiency ratio and the proportion of lysine in the protein. In mixtures of these protein sources, a lysine content of 200 mg. or more per g. of nitrogen appears necessary to obtain a protein efficiency ratio in the high-quality range of 2 or higher.

In all of the cases tested, the fat and protein contents of the rat livers were well within accepted limits (Elvehjem, 5). However, it was noted that the livers of rats on the casein diet contained slightly more protein and less fat than the livers of rats on the bread diets, whether the bread was supplemented with lysine or not. In other words, it appears that the addition of free lysine increased the protein quality of the bread diets as measured by over-all body growth, but did not significantly alter differences in liver composition resulting from feeding the different proteins and protein mixtures studied in this series of experiments.

Rosenberg and Rohdenburg (10) noted that supplementation with as high as 1.0 part L-lysine monohydrochloride per 100 parts flour did not increase the protein efficiency ratio of white bread diets much above 2, the efficiency obtained by supplementation with 0.25 part of the amino acid salt. However, the results of the present study showed that when protein bread diets were supplemented with even lower proportions of lysine, efficiency ratios as high as 2.6 were attained.

This would indicate that the protein supplement in these breads must increase the potential quality rating which can be obtained by further supplementation with lysine. This may be the result of the greater proportion of total essential amino acids in the mixed protein of the soy-milk and milk-lactalbumin-soy breads. The milk proteins have, in effect, made the breads more concentrated in essential amino acids, thus increasing the proportion of food protein which can be utilized for tissue protein synthesis. Lysine added to this *mixed* protein improves the balance of amino acids, and even greater protein efficiency is thereby obtained.

Acknowledgments

The authors wish to thank L. R. Di Maio, Experimental Station, E. I. du Pont de Nemours & Co., for his analytical assistance in the lysine determinations on the breads used in this study.

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The Role of Amylase Activity in the Rapid Increase of the Maltose Content of Wheat Flour Doughs during Mixing[1]

J. W. LEE[2] AND W. F. GEDDES

ABSTRACT

The maltose content of a freshly mixed, unyeasted flour-water dough was 1.41% (14% moisture basis), as compared with 0.12% in the Southwestern Baker's Patent flour from which it was made. No increase in maltose occurred in a similar dough made from ethanol-inactivated flour; addition of a dialyzed aqueous extract of the original flour yielded a dough containing 1.29% maltose. Ball-milling the inactivated flour markedly enhanced the increase in maltose content of unyeasted doughs to which enzyme extract was added. These experiments justify the conclusion that the very rapid increase in the maltose contents of regular sponges and doughs during mixing is due to the action of beta-amylase upon the susceptible starch in the flour.

The concentration of maltose in a yeasted wheat flour dough is dependent upon three main factors: the amount present in, or added to, the flour; the amount which is produced by the action of beta-amylase on susceptible starch; and the rate at which maltose is utilized by yeast. The amount of maltose in wheat flour has been determined recently by Koch, Geddes, and Smith (8) and Williams and Bevenue (14), who found concentrations of 0.07% and 0.05% respectively. The production of maltose by the action of beta-amylase on susceptible starch has been shown to be more dependent upon the quantity of available starch than on the concentration of beta-amylase in the dough (3). That mechanical injury to starch granules can affect their properties and susceptibility to amylase attack has been shown in a number of studies (2,5,6,10,15).

Koch, Smith, and Geddes (9) and Lee, Cuendet, and Geddes (11) observed that the maltose content of a straight dough or sponge, immediately after mixing, was some 10 to 15 times greater than that of the flour from which it was made, when expressed on the same moisture basis. The maltose concentration increased until the glucose and fructose levels had fallen to low values; thereafter, the concentration of maltose in the straight dough or sponge decreased with time. These results are in accord with reports in the literature that commercial baker's yeast ferments glucose and fructose in preference to maltose

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(1,4,12). When these sugars become limiting, the yeast becomes adapted to the fermentation of maltose.

The object of the present study was to determine whether the sudden increase in maltose concentration upon dough formation can be ascribed to enzyme action. Doughs were made with normal flour, with flour in which the amylase enzymes had been inactivated and with flour which had been ball-milled to increase the content of susceptible starch.

Materials and Methods

A Southwestern Baker's Patent flour (protein 11.8%, ash 0.47% on a 14% moisture basis) was used in all experiments.

Preparation of Inactivated Flour. One hundred grams of flour were boiled with 250 ml. absolute ethanol on a steam bath, and the heating continued until the ethanol had evaporated. During the evaporation, the flour was continuously mixed to avoid separation of the lipids. The flour was placed in a vacuum desiccator containing concentrated sulfuric acid to remove the last traces of ethanol. It was assumed that this treatment with boiling ethanol inactivated all the beta-amylase in the flour.

Preparation of Amylase Extract. An enzyme preparation was made as follows:

Flour (250 g.) was extracted with 1,000 ml. water at room temperature for 2 hours. The mixture was centrifuged and the supernatant placed in cellophane sausage casings (Visking 1⅝-in. diam.) and exhaustively dialyzed against distilled water for 4 days at 5°C. The liquid in the casing was then pervaporated until the volume was reduced to about 50 ml. Paper chromatographic estimation showed that maltose was not present in the enzyme preparation.

Ball-Milling of Flour. Samples of the ethanol-inactivated flour were ball-milled for 72, 192, and 384 hours, respectively. The ball-mill used was of 4,000-ml. capacity and contained 60 porcelain balls ranging in diameter from 1.0 to 2.5 cm.

Preparation of Doughs. A yeasted dough was prepared by mixing 30 g. flour and 0.86 g. fresh compressed baker's yeast with the appropriate amount of distilled water in a micromixer (National Manufacturing Co., Lincoln, Nebr.) for 3 minutes. Unyeasted doughs were made in the same manner from normal flour and inactivated flour. A further series of unyeasted doughs was prepared from 30-g. samples of inactivated flour which had been ball-milled for 0, 72, 192, and 384 hours, respectively, and to which were added 10-ml. portions of the enzyme

preparation.[3] The temperature of all doughs was 30° ± 0.5°C.

Determination of Sugars. Glucose, fructose, sucrose, and maltose were determined in the flour, yeast, enzyme extract, and doughs by the method of Koch *et al.* (9), as modified by Lee, Cuendet, and Geddes (11). This method involved inactivation of the yeast and flour enzymes, extraction, paper chromatographic separation and determination of individual sugars by the method of Dubois *et al.* (3). The samples of dough were taken for analysis immediately after mixing.

Results and Discussion

The results of sugar determinations on the original flour, yeast, yeasted and unyeasted doughs prepared from the original flour are given in Table I. Analyses of the doughs were commenced as soon as dough-mixing was completed.

It is apparent that the factor(s) responsible for the sudden increase in maltose concentration upon dough-mixing are present in the flour and not in the yeast. The yeast is, however, responsible for inversion of the sucrose and for the production of glucose and fructose by hydrolysis of the glucofructans of the wheat flour.

Maltose determinations on unyeasted doughs prepared from flour treated in various ways are recorded below:

Flour Component of Dough	*Maltose*[a]
	%
Normal flour	1.41
Ethanol-inactivated flour	0.15
Ethanol-inactivated flour plus enzyme preparation	1.29
Ethanol-inactivated flour ball-milled 72 hours, plus enzyme preparation	1.57
Ethanol-inactivated flour ball-milled 192 hours, plus enzyme preparation	1.84
Ethanol-inactivated flour ball-milled 384 hours, plus enzyme preparation	2.18

[a] Means of duplicate determinations (14% moisture basis) made on doughs immediately after mixing.

The maltose content of the unyeasted dough made from inactivated flour was in good agreement with that of the original flour, but inclusion of the enzyme extract increased the maltose content to 1.29%. The extent of this sudden initial increase in maltose concentration was made greater by ball-milling the flour, a treatment which increases the quantity of susceptible starch (6,10).

These experiments justify the conclusion that the very rapid production of maltose observed during the mixing of sponges and doughs

[3] The quantity of enzyme preparation added to these doughs represents an excess. This excess was added to allow for potential losses during preparation of the enzyme and to magnify any differences between inactivated flour and inactivated flour plus enzyme preparation.

TABLE I

SUGAR LEVELS IN FLOUR, YEAST, AND IN YEASTED AND UNYEASTED DOUGHS IMMEDIATELY AFTER MIXING AT 30°C.

SYSTEM ANALYZED	SUGAR CONCENTRATION [a] (14% MOISTURE BASIS)			
	Glucose	Fructose	Sucrose	Maltose
	%	%	%	%
Flour	0.02	0.05	0.15	0.12
Yeast	0.00	0.00	0.00	0.00
Yeasted dough	0.40	0.61	0.00	1.47
Unyeasted dough	0.04	0.06	0.15	1.44

[a] Means of duplicate determinations.

is due to the action of beta-amylase on the susceptible starch which is present in commercial flours. Following the utilization of the limited quantities of very susceptible starch which are present, the subsequent rate of maltose production would not be as great, and would be largely determined by the rate at which further susceptible starch was produced by the action of alpha-amylase. This hypothesis explains the findings of Koch *et al.* (9) and Lee *et al.* (11). It is in accord with the observation of Kneen and Sandstedt (7) and of Stamberg and Bailey (13) that flour milled from sound wheat contains adequate amounts of beta-amylase and with the views of Blish, Sandstedt, and Kneen (2) that the rate of autolytic maltose production is determined primarily by the concentration of susceptible starch.

Acknowledgment

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A NOTE ON THE EFFECTS OF NEUTRAL POLYSACCHARIDES ON DOUGH MIXING PROPERTIES[1]

C. A. WILHAM, R. J. DIMLER, AND F. R. SENTI

This note presents the results of limited studies on the effect of neutral polysaccharides on dough mixing properties. These experiments are of particular interest because they demonstrate an alternate approach to the study of the role of protein-carbohydrate interactions in flour and dough quality. The more direct approach of using natural polysaccharides of wheat flour is seriously handicapped by the difficulty of isolating flour polysaccharides (especially the insoluble pentosans) in pure form. The use of a variety of well-defined, pure, neutral polysaccharides provides an opportunity for conducting studies of carbohydrate interaction in which evidence can be obtained on the role of their chemical structure and of molecular size. The fact that these studies will not be extended by us makes it desirable to present the known results as a guide and stimulus to other research workers in this field.

Evidence of unique and contrasting differences in the nature of the polysaccharide-gluten interaction was obtained in comparative studies with starch products and dextrans. The latter are neutral

[1] Manuscript received September 8, 1958. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

polymers of glucose formed by microbial or enzyme action on sucrose (1).

Farinograph mixing curves reproduced in the figures provide a quantitative basis for comparing the effects of the polysaccharides. Additions were made to a Lee (hard red spring) wheat flour of 70% extraction, using a constant total weight of ingredients (flour, additive if any, and water). The polysaccharides were "native" or high-molecular B-512 dextran,[2] native B-742 dextran (1), "clinical" or low molecular B-512 dextran (4), and wheat starch. Native B-512 has a molecular weight of millions (4); clinical B-512, produced from the native dextran by limited acid hydrolysis and fractional precipitation, has a molecular weight of about 75,000.

The mixing curve for Lee flour is reproduced in Fig. 1. Addition of 2% native B-512 dextran, based on the weight of flour, increased the absorption value of the flour from 59.7 to 72.5% (Fig. 2), or to 70.7% if the absorption is calculated on the basis of weight of flour plus dextran.

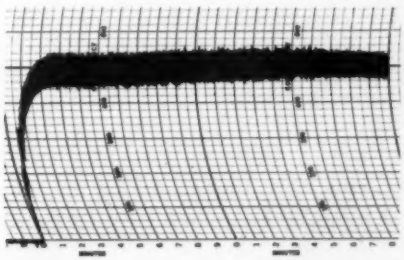

Fig. 1. Farinograph pattern for Lee (hard red spring) wheat flour of 70% extraction with a moisture content of 12.75% and a protein content on an "as-is" basis of 12.2%. Mix: 296 g. flour and 183 ml. water.

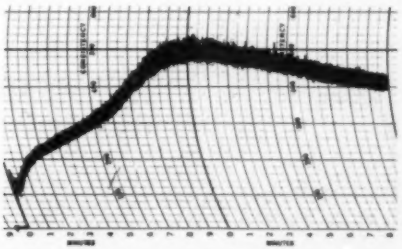

Fig. 2. Effect on farinograph pattern when 2% of native B-512 dextran is added to Lee wheat flour. Mix: 270 g. flour, 6.5 g. B-512 dextran, and 202.5 ml. water.

[2] A dextran is designated by the number for the strain of microorganism used in its synthesis, in this case *Leuconostoc mesenteroides* NRRL B-512 (see ref. 1).

A major factor contributing to this large increase in absorption value appears, on the basis of present evidence, to be polysaccharide-protein interaction. One indication is the change in the shape of the mixing curve (Fig. 2 compared with Fig. 1) which reflects an effect of dextran on mixing tolerance, as well as on hydration during dough development. A further demonstration of an adverse effect of native B-512 dextran on the gluten complex was provided by kneading a gluten ball in a 5% solution of the dextran. The gluten ball partially disintegrated, suggesting that the dextran altered the gluten properties directly, for example, through protein-carbohydrate interaction or by displacement of one or more components from the original gluten complex. Similar solutions of the other polysaccharides, for which mixing data are presented, failed to have this effect on a gluten ball.

An alternative explanation for the high absorption value would be a high level of water-binding by the native B-512 dextran. While this possibility cannot be excluded completely, calculations show that the dextran would have to absorb about 6.2 ml. water per g., compared with 0.62 ml. per g. for the flour. In addition, large differences would have to exist in the water-binding capacity among the polysaccharides included in this study. At present there is no basis for anticipating such differences. The effect of a solution of native B-512 dextran on the gluten ball also lacks a ready explanation on the basis of water-binding alone.

Differences in dextran structure influenced the extent to which flour mixing properties were altered. Adding 2% of the more highly branched native dextran B-742 (1) only increased the absorption value of the flour from 59.7 to 64.3% (or 62.6% based on flour plus dextran), and it had much less influence on the shape of the mixing curve (Fig. 3) than did native B-512 dextran.

Further evidence of the influence of polysaccharide structure is pro-

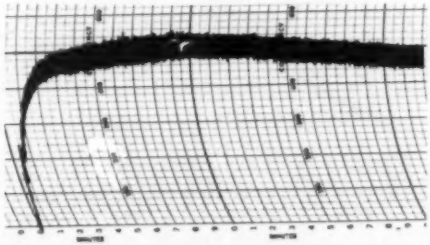

Fig. 3. Effect on farinograph pattern when 2% of native B-742 dextran is added to a hard red spring wheat flour. Mix: 283.5 g. flour, 6.5 g. B-742 dextran, and 189.0 ml. water.

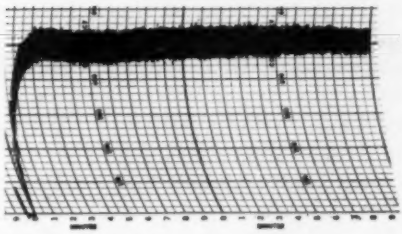

Fig. 4. Effect on farinograph pattern when 2% of wheat starch is added to a hard red spring wheat flour. Mix: 290.0 g. flour, 6.7 g. wheat starch, and 182.3 ml. water.

vided by the results with wheat starch (Fig. 4). The flour absorption value after addition of 2% starch was 60.7% (or 59.1% on the basis of flour plus starch). Apparently the starch was nearly inert, having equal or less water absorption than the flour. Essentially the same mixing behavior was obtained on addition of 2% pregelatinized wheat starch prepared by vigorous mechanical agitation of the pasted starch, followed by dehydration with alcohol (2). The result with pregelatinized starch indicates that water solubility or ability to form a paste at room temperature does not account for the greater effect of the native dextrans on absorption value.

A different type of interaction was observed when clinical dextrans having an average molecular weight of about 75,000 were used. As shown in Fig. 5, the addition of 2% clinical B-512 dextran to the flour

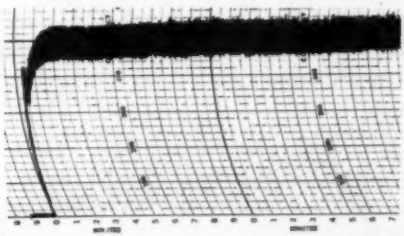

Fig. 5. Effect on farinograph pattern when 2% of clinical B-512 dextran is added to a hard red spring wheat flour. Mix: 296.9 g. flour, 7.2 g. B-512 dextran, and 175.0 ml. water.

decreased the absorption value of the flour from 59.7 to 56.2% while causing no significant change in the mixing curve. In this case the clinical dextran appears essentially to be equivalent to water in having a lubricating or softening action on the dough. Thus a 296-g. portion of Lee flour required 183 ml. of water to produce the curve in Fig. 1. An almost identical weight of flour (296.7 g.) required only 175.1 ml.

of water to give the same consistency when 7.2 g. of clinical B-512 dextran was added (Fig. 5). Therefore, the 7.2 g. of clinical dextran was equivalent to 8 ml. of water in the mix, rather than acting as an inert diluent with no effect on the flour-to-water ratio.

The marked difference between native B-512 dextran and clinical B-512 dextran in the effect on absorption value and mixing characteristics appears to be related to molecular size of the polysaccharide. Except for size, the molecular structure of the clinical dextran differs very little from that of the native dextran on the basis of existing data (3,4). Further evidence that molecular size is a controlling factor in the case of the dextrans was provided by comparative data on the addition of other structurally different clinical dextrans to flour. Clinical B-742 dextran (5) caused the same decrease in absorption value as did clinical B-512 dextran, in contrast to the difference between their corresponding native dextrans.

While the decrease in absorption value can be associated with molecular weight for some carbohydrates, other factors must also be operative. Outside the dextran class several carbohydrates differing in molecular size and structure gave a diversity of results. Sucrose (molecular weight 342) and an acid-hydrolyzed waxy maize starch (molecular weight about 16,000) caused similar if not so extensive decreases in absorption value as the clinical dextrans. In contrast, dextrose produced no apparent change in the mixing curve, while a Naegeli dextrin[3] with molecular weight about 4,000 caused a slight increase in absorption but no variation in the mixing curve.

In addition to comparisons of different polysaccharide samples, limited comparisons of wheat flours were made. Variations were noted in the extent to which native B-512 dextran influenced the mixing

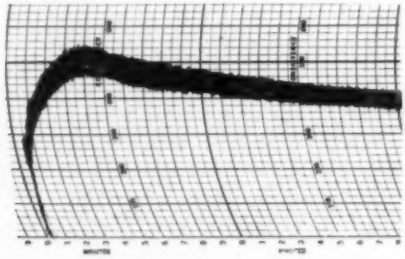

Fig. 6. Farinograph pattern for Red Chief (hard red winter) wheat flour of 63% extraction with a moisture content of 12.9% and a protein content on an "as-is" basis of 12.1%. Mix: 283 g. flour and 196 ml. water.

[3] Prepared by R. L. Lohmar of this Division by steeping defatted white potato starch for 6 months in periodic changes of 15% sulfuric acid, followed by dissolution in hot water and crystallization from aqueous methanol, yielding a product readily soluble in water at room temperature.

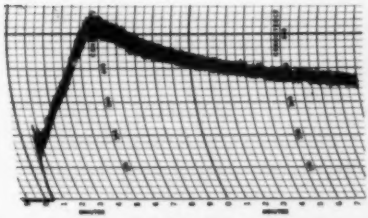

Fig. 7. Effect on farinograph pattern when 2% of native B-512 dextran is added to Red Chief wheat flour. Mix: 264 g. flour, 6.3 g. B-512 dextran, and 209 ml. water.

properties. Red Chief wheat, which has poor mixing tolerance (Fig. 6), underwent some retardation of dough development, but relatively little further decrease in mixing tolerance (Fig. 7). However, the increase in absorption from 67 to 77% indicated a protein-carbohydrate interaction comparable to that of the Lee flour.

The observations of gluten-dextran interaction are of potential importance, not only to the fundamental study of gluten properties but also to the technology of dough and bread. Interaction of native dextran, particularly the B-512 types, would provide an avenue for investigating factors influencing extensibility and gas retention of gluten. The "lubricating" or "softening" action of clinical dextrans might be utilized in studies directed toward improvement in texture (softness) and toward retardation in firming or staling of bread.

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Cereal Chemistry

ERRATUM

Cereal Chemistry, Vol. 36, No. 3

PAGE 283, LINKO AND MILNER:

Paragraph 4, line 22 from top of page should read:
equivalent to 100 mg. of germ and 200–500 mg. of grain was sufficient . . .

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, of fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

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Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of author's names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **45**: VII–CCLV (1951).

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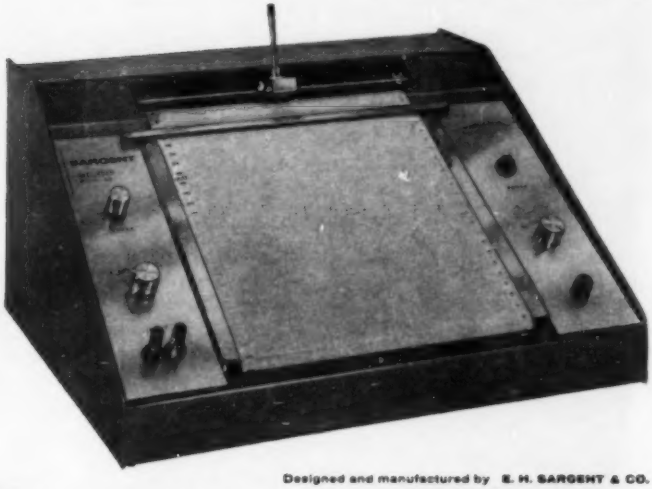

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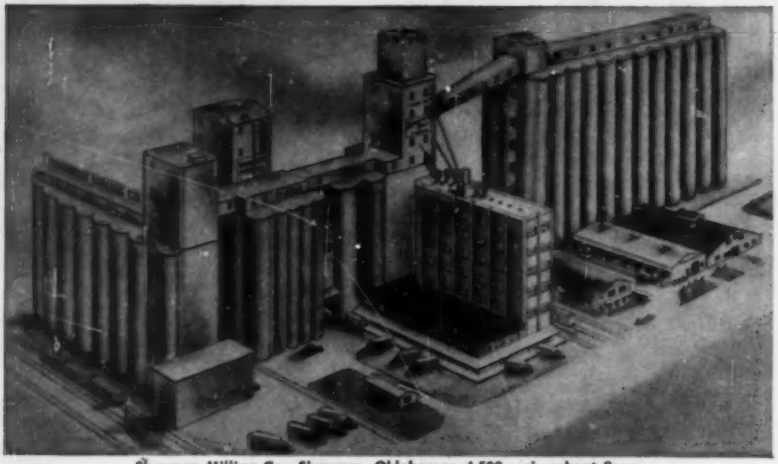

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